636.2.082

392. SEASONAL VARIATIONS IN THE SALES OF CALVES IN DIFFERENT PARTS OF GREAT BRITAIN, 1943–1947

BY R. PHILLIPS
Department of Animal Health, University College of Wales, Aberystwyth
E. H. BROWN AND J. L. DAVIES
Department of Geography and Anthropology, University College of Wales, Aberystwyth

(With 5 Figures)

As an outcome of investigations into the seasonality of calf sales in Wales (Phillips & Davies (1) and Phillips, Davies & Brown (2)) and also into the calvings of in-calf heifers (Philips (3)), it was thought desirable to study the disposal of the annual calf crop of Great Britain during the five years 1943–7.

A description of the disposal of the calf crop in this country during the pre-war years was made by Wood & Newman (4). Others (5, 6, 7) from milk-recording data have computed the annual 'wastage' in dairy herds as well as the seasonality of calvings. Farm surveys (8, 9) have also been made for the same purpose.

Recently Marley (10), in a comprehensive paper based on official statistics, has measured the annual inflow and outflow of cattle in the national herd of Great Britain and has taken into account the importations of cattle from Eire and Northern Ireland. The Ministry returns of calf births are not in agreement with Miss Marley's estimates, whilst her estimated quarterly rates of calving of cows and heifers are not in accord with the estimates based on the National Milk records, A.I. records, Milk Production records (M.M.B.) and the sales of calves (Ministry of Food). It is believed that Miss Marley's estimate of the annual replacements in the national dairy and breeding herd is too low.

In this paper an attempt is made to describe the disposal of the annual crop and to obtain an alternative measure of the inflow and outflow through the herds of the country. The estimations and calculations are based on the following statistics so as to cover the whole or part of the five-year period 1943–7:

(1) The 'calf births' in the Ministry's quarterly returns.
(2) The 'calf sales' to the Ministry of Food.
(3) The 'quarterly returns' of the Ministry of Agriculture.

(1) *Calf births* (*Ministry's quarterly returns*, 1945–7).

The births of calves in England and Wales have been recorded in the quarterly returns of the Ministry for each month since June 1944, and these records have been examined for three years 1945–7 so as to coincide with the year's calf-sale data (1943–7). Up to the present time it has not been possible to obtain the calf-births data for Scotland.

(2) *Calf sales* (*Ministry of Food*)

The monthly sales of calves (bobbies and veals) have been obtained for each collecting centre in the country for the five years 1943–7. Groups of parishes have been allocated to each collecting centre, from which supplies are drawn at weekly or fortnightly intervals.

The Chairmen of the local auctioneers acting on behalf of the Ministry of Food have endeavoured, on a 'give and take' system, to allocate the collecting centres to their respective counties in such a way that centres allocated near to county boundaries are drawing their supplies from more than one county and are arranged, territorially at any rate, so as to balance one another in the matter of county returns.

The calves (mainly males) sold for slaughter to the Ministry of Food, are, of course, the surplus products of the dairy farms; consequently, the data provide useful evidence of the distribution of the calvings on dairy farms in small localities consisting of a few parishes, and also on a county basis. The calf-sales data do not give any information of the calving rates in areas concerned with the rearing of cattle, and where an area is concerned with both the dairying and the rearing systems, the calf-sale evidence can only give information for the dairying portion, and even for that a caution must be given because of the transfer of calves from dairying farms at certain times of the year. However, in west Wales, where the evidence was examined in detail for the period 1943–6, the extent of this transfer was very small.

The seasonality of calf sales in dairying areas is therefore an extremely useful check on the calf-births data in the returns of the Ministry of Agriculture, and is of considerable value in defining the dairying and rearing areas of this country.

(3) *Quarterly returns* (*Ministry of Agriculture*)

These returns give the number of cattle in various categories at certain fixed times of the year, viz. the 4th day of the months of March, June, September and December. The statistics do not give the rate of flow of the animals through the national herd, although certain computations make it possible to measure the flow from them (cf. Smith (11), Marley (10) and Phillips (3)).

It is possible, however, to measure from these statistics the size of the breeding stock in the national herd at certain fixed times; during the years 1943–7 this has remained fairly stationary. From these statistics also can be measured the quarterly calvings of the in-calf heifers and therefore the rate of replenishment of the national herd; and consequently a measure of the outflow from that herd can be obtained.

The recorded slaughterings (Ministry of Food) of various categories of cattle (steers, heifers, cow heifers, cows and bulls) also provide information on the flow from the herd.

Comparison of annual calf births and the annual calf crop

The calf births per month for the three years 1945–7 are obtained from the quarterly returns of the Ministry. The annual 'calf crop' for the purposes of this paper is the sum of the annual number of calves sold for slaughter to the Ministry of Food and of those recorded as cattle 'under one year old' on 4 June. These have been found to give practically the same percentages as the mean of the quarterly returns and consequently have been used throughout.

From Table 1 it can be calculated that in England the annual 'calf crop' is on the average 86% of the calf births and 81% for Wales. The calf crop is less than the calf births for several seasons, but mainly because of casualties in young calves and also because it is thought that in the past farmers have been in the habit of entering this group as 'cattle over 1 year old and under 2' in the Ministry returns when they attain the age of 10–10½ months.

Table 1. *Number of calves sold and reared, etc., and comparative annual figures with* 1943 = 100

Country	Year	No. of calves sold	Cattle under 1 year old 4 June	Annual calf crop	Calf births	Calf sales	Cattle under 1 year old	Calf crop	Calf births
England	1943	1,080,770	1,169,005	2,249,775	—	100·0	100·0	100·0	—
	1944	1,090,241	1,181,506	2,271,747	—	101·0	101·0	101·0	—
	1945	1,152,937	1,154,059	2,306,996	2,596,981	106·7	99·0	102·2	100·0
	1946	1,147,873	1,127,733	2,275,606	2,664,458	106·2	96·5	101·0	102·2
	1947	1,042,665	1,151,527	2,194,192	2.541,977	96·5	98·5	97·5	98·0
Wales	1943	127,636	199,418	327,054	—	100·0	100·0	100·0	—
	1944	136,854	199,541	336,395	—	107·2	100·0	102·5	—
	1945	155,544	187,048	343,592	411,984	122·0	93·7	104·5	100·0
	1946	169,325	172,890	342,275	428,135	132·7	86·7	104·5	104·0
	1947	148,640	165,988	314,628	393,898	116·5	83·1	96·2	96·0
Scotland	1943	116,820	286,033	402,853	—	100·0	100·0	100·0	—
	1944	119,839	292,910	412,749	—	102·6	102·4	102·1	—
	1945	123,135	286,447	409,582	—	105·4	100·0	101·4	—
	1946	133,924	288,455	422,379	—	114·7	101·0	104·7	—
	1947	128,813	287,581	416,394	—	110·2	100·6	103·0	—
Great Britain	1943	1,325,226	1,654,456	2,979,682	—	100·0	100·0	100·0	—
	1944	1,346,934	1,673,957	3,020,891	—	101·6	101·0	101·0	—
	1945	1,431,616	1,627,554	3,059,170	—	108·0	98·5	102·5	—
	1946	1,451,182	1,589,078	3,040,260	—	109·5	96·0	102·0	—
	1947	1,320,118	1,605,096	2,925,214	—	100·0	98·0	98·0	—

Wales shows the greatest increase in the comparative annual calf sales, but all show a sharp drop in 1946–7. Wales also shows a sharp decline in cattle under 1 year old. The differences in the annual calf crops are well within the limits of 10%.

Over one and a third million calves are slaughtered annually in Great Britain, representing an average of 45·7% of the annual calf crop. Of the total slaughtered, England sells anually 1·1 million calves, Wales nearly 150,000 and Scotland 125,000, representing 48·8, 44·4 and 30·1% respectively of the annual calf crop in each of the three countries.

The highest number of calves sold was reached in 1946, except in England, where the peak was reached in 1945. The highest percentage sales were reached in 1946 in all cases.

Calf births and the calf crop as a percentage of cows in milk and cows in calf but not in milk

The relationship between the recorded calf births and the annual calf crop as a percentage of the cow population gives information on the fertility rate of the national herd in this country.

In Table 2 the average calf crop as a percentage of the cow population on 4 June shows remarkable regularity. The average figure for Great Britain of 92·2% is nearly identical with that quoted by the New Zealand Dairy Board in its report for 1946–7, where the calvings as a percentage of the total cows is given as 92·7%. Apart from Scotland there was a gradual improvement from year to year in the percentage calf crop from 1943 to 1946, but there was a sharp decline from 1946 to 1947.

It is seen that the calf births are greater than the cow population given in the 4 June returns of the Ministry. This is because the in-calf heifers are excluded from the total breeding herd, although a high proportion of those recorded on 4 June would calve by the end of the year and consequently would tend to make the percentage calf births above 100% as above.

Table 2. *The annual calf births and calf crop as a percentage of cows* (4 *June*)

Country	Year	Cows and heifers in milk: and cows in calf not in milk (4 June)	No. of calf births	Annual calf crop	Calf births as % of cows	Calf crop as % of cows
England	1943	2,466,558	—	2,249,775	—	91·2
	1944	2,475,749	—	2,271,747	—	91·7
	1945	2.419,801	2,596,981	2,306,996	107·0	95·3
	1946	2,405,465	2,664,458	2,275,606	110·5	94·6
	1947	2,395,185	2,541,977	2,194,192	106·2	91·6
					Average	92·8
Wales	1943	353,717	—	327,054	—	92·7
	1944	366,551	—	336,395	—	91·7
	1945	365,842	411,984	342,592	112·2	93·6
	1946	368,986	428,135	342,275	116·0	92·7
	1947	368,316	393,898	314,628	107·0	85·4
					Average	91·2
Scotland	1943	450,978	—	402,853	—	89·3
	1944	448,782	—	412,749	—	91·9
	1945	463,407	—	409,582	—	88·3
	1946	467,252	—	422,379	—	90·4
	1947	469,650	—	416,394	—	88·6
					Average	89·7
Great Britain	1943	3,270,253	—	2,979,682	—	91·1
	1944	3,291,082	—	3,020,891	—	91·8
	1945	3,249,050	—	3,059,170	—	94·1
	1946	3,241,703	—	3,040,260	—	93·8
	1947	3,233,151	—	2,925,214	—	90·5
					Average	92·2

Calf sales as a percentage of the 'calf crop'

The calf sales as a percentage of the annual calf crop have been calculated for each county, and the 5 year averages have also been obtained. The various counties in England, Wales and Scotland have been grouped in accordance with the variations in the average percentages, as shown in Table 3.

The wide variations between the groups in Table 3 no doubt indicate differences in the relative importance of the systems—dairying, or cattle rearing and beef production—in different parts of the country. Many counties sell nearly two-thirds of the annual calf crop and therefore do not rear all their heifer calves for herd replacement purposes, whilst in others less than one-third of the annual calf crop is sold for slaughter.

The following map (Fig. 1) based on the 5-year (1943–7) average percentage illustrates the various groups of counties.

(1) *England*

It is seen from Fig. 1 that seven counties in England sell over 60% of the annual calf crop and another seven counties show a disposal of 50% and over. These are the intensively dairying counties which have remained unaltered during the 5 years under review. Two groups, however (Nos. 5 and 6), sell less than 30% of the annual calf crop, and apart from Herefordshire on the Welsh borders, the remainder of these are in the eastern half of England, mainly along the east coast to the north of the Wash. Cattle rearing, therefore, remains an important industry in some of the arable areas of the east coast (e.g. Lincolnshire). Estimated on the calf-sales basis dairy farming is shown to occupy the greater portion of England.

Table 3. *Calf sales as a percentage of the annual calf crop for each county (counties arranged in groups)*

County	1943	1944	1945	1946	1947	1943–47 Average
England:						
		Group I, over 60%				
Cheshire	67·0	67·0	70·0	71·5	69·6	69·0
Lancashire	58·0	59·0	61·7	66·4	63·8	61·8
Stafford	65·7	65·0	67·0	68·1	65·6	66·3
Wiltshire	71·0	71·5	73·0	74·0	69·0	71·7
Hampshire	58·1	60·3	62·0	59·3	57·0	59·3
Isle of Wight	56·5	60·0	67·7	65·4	62·1	62·3
Dorset	71·2	71·5	72·3	69·7	68·2	70·6
Somerset	68·5	68·0	68·7	69·2	65·5	67·6
					Average	66·1
		Group II, 50–60%				
Salop	55·0	53·5	55·4	58·0	55·6	55·6
York—West Riding	50·7	58·1	48·7	52·3	50·2	50·0
Westmorland	50·0	49·5	53·2	59·0	53·0	52·9
Derby	57·7	55·8	58·5	60·5	58·4	58·2
Surrey	56·5	54·1	61·4	59·0	53·6	56·9
Sussex, east and west	53·0	55·5	58·7	56·6	51·6	55·1
Berkshire	58·1	58·7	60·5	57·7	55·5	58·1
					Average	55·2
		Group III, 40–50%				
Warwick	53·2	47·6	49·3	47·6	46·7	48·9
Essex	47·3	49·5	51·7	51·0	47·5	49·4
Gloucester	54·3	49·5	50·0	46·8	43·2	48·8
Oxford	46·5	45·2	45·7	45·0	40·8	44·6
Kent	44·8	48·6	51·6	49·1	44·5	47·7
Leicester	48·7	44·7	47·0	45·3	42·5	45·6
Herts and Middlesex	48·8	50·1	51·5	47·6	44·1	48·4
Cambridge	33·0	38·7	43·6	43·8	42·0	40·2
					Average	46·4
		Group IV, 30–40%				
Norfolk	30·0	35·0	39·8	37·4	34·2	35·3
Cumberland	29·7	28·7	33·5	38·4	35·4	33·1
Cornwall	32·7	40·0	41·6	40·5	36·9	38·3
Devon	32·0	35·7	35·8	33·4	30·1	33·4
Durham	39·0	32·7	37·2	39·9	38·0	37·4
Suffolk	31·1	34·7	39·0	35·3	31·4	34·3
Worcester	47·6	41·0	40·4	39·8	33·4	40·4
					Average	36·0
		Group V, 20–30%				
Northumberland	21·6	19·4	24·1	26·6	24·4	23·2
York—North Riding	28·3	23·3	29·2	32·7	31·9	29·1
Hereford	17·7	21·5	21·3	19·1	19·7	19·9
Northampton	35·8	29·2	30·0	27·6	24·9	29·4
Nottingham	21·4	21·3	27·0	26·6	25·9	24·4
					Average	25·2
		Group VI, under 20%				
Huntingdon	9·5	12·4	13·5	14·6	16·4	13·3
Rutland	1·0	0·7	2·2	2·3	2·8	1·8
York—East Riding	12·0	11·0	12·3	14·4	14·2	12·7
Lincoln	7·3	8·4	11·5	13·4	12·5	10·6
					Average	9·6
Wales:						
		Group I, over 60%				
Carmarthen	61·3	63·0	66·7	69·5	68·6	65·6
		Group II, 50–60%				
Glamorgan	52·7	54·0	56·7	59·6	56·4	55·9
Cardigan	41·6	44·5	51·7	56·5	56·2	50·1
					Average	53·0

Table 3 (*continued*)

County	1943	1944	1945	1946	1947	1943–47 Average
		Group III, 40–50%				
Denbigh	34·7	36·2	42·5	47·0	45·1	41·1
Monmouth	46·0	44·8	47·4	49·4	46·4	46·8
Pembroke	36·9	40·6	47·1	51·2	49·0	45·0
					Average	44·3
		Group IV, 30–40%				
Caernarvon	30·6	30·9	37·0	45·7	42·3	37·3
Flint	33·8	33·8	38·2	42·1	40·2	37·6
					Average	37·4
		Group V, 20–30%				
Anglesey	14·0	16·1	22·0	34·0	31·1	23·4
Brecon	28·7	29·0	30·6	29·0	29·3	29·3
Merioneth	20·1	21·6	30·9	36·4	31·4	28·1
Montgomery	17·2	19·1	22·0	23·8	24·2	21·3
					Average	25·5
		Group VI, under 20%				
Radnor	4·7	5·9	7·4	7·5	5·1	6·1
Scotland:						
		Group I, above 60% (nil)				
		Group II, 50–60%				
Lanark	55·3	56·1	—	—	—	55·7
Renfrew	58·5	58·1	—	—	—	58·3
					Average	57·0
		Group III, 40–50%				
Ayr	48·0	49·1	—	—	—	48·5
Midlothian	42·7	40·8	—	—	—	41·7
Wigtown	50·1	47·3	—	—	—	48·7
West Lothian	39·8	41·4	—	—	—	40·6
Stirling	39·8	41·4	—	—	—	41·6
Kirkcudbright	40·0	41·0	—	—	—	40·5
					Average	43·6
		Group IV, 30–40%				
Dumfries	39·6	37·9	—	—	—	38·7
Dumbarton	33·1	32·5	—	—	—	32·8
					Average	35·7
		Group V, 20–30%				
Argyle	22·0	22·3	—	—	—	22·1
Bute	25·3	24·4	—	—	—	24·8
Fife	24·5	23·7	—	—	—	24·1
					Average	23·7
		Group VI, under 20%				
Aberdeen	3·4	5·0	—	—	—	4·0
Angus	13·0	13·3	—	—	—	13·1
Banff	2·8	2·4	—	—	—	2·6
Berwick	6·3	5·7	—	—	—	6·0
Caithness	2·5	3·3	—	—	—	2·9
East Lothian	10·2	12·5	—	—	—	11·3
Inverness	3·6	3·3	—	—	—	3·4
Kincardine	3·6	4·0	—	—	—	3·8
Moray and Nairn	8·1	8·3	—	—	—	8·2
Selkirk	10·0	11·9	—	—	—	10·9
Perth	5·2	5·3	—	—	—	5·2
Ross and Cromarty	1·7	3·4	—	—	—	2·5
Roxburgh	12·4	15·7	—	—	—	14·0
Sutherland	2·9	2·6	—	—	—	2·8
					Average	6·2

(2) *Wales*

Carmarthenshire has the highest percentage calf sales in Wales. This county sells two calves out of every three, and its annual total of sales is nearly one-third of that for the whole of Wales. There are five counties in Wales selling less than 30 % of the calf crop and these are the rearing counties, although Anglesey and Merioneth had overreached this figure by 1946 and 1947.

Flint is known to be an intensive dairying county, although it occupies only a medium position in the above groupings and percentages. This is probably due to the fact that it is

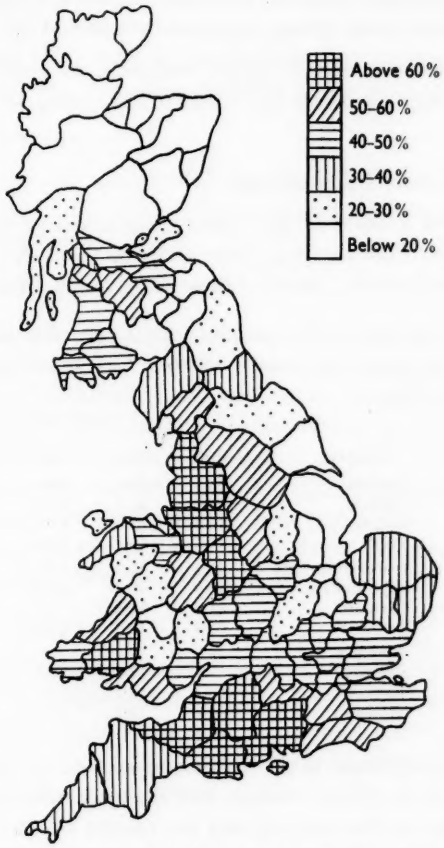


Fig. 1. Calf sales as a percentage of calf crop (1943–7). Counties of England and Wales.

a small narrow county with convenient collecting centres situated in the adjoining counties of Cheshire and Denbighshire, through which many of the surplus calves are no doubt sold.

The Radnorshire percentage figure is low because the county has remained true to its traditional system of mixed farming and cattle rearing, whereas Brecon shows practically no annual changes because the county had already split itself into two well-defined portions prior to 1943, viz. the new dairying areas and the old-established rearing areas.

Several counties in Wales have shown rapid increases in the annual percentage calf sales,

notably Anglesey, Cardigan and Denbigh, and these counties are in a different group in 1947 compared with their respective positions in 1943.

(3) *Scotland*

The map of Scotland based on 2 years only (1943–4) (Fig. 1) shows a well-defined division of the country into the intensive dairying areas of the south-west with varying percentages above 40%; the rearing areas of the south-east; and all the counties to the north of the line from the Forth to the Clyde with less than 20% calf sales. In these northern counties of Scotland the calf sale is a low percentage of the annual calf crop, and in most of them the figures are extremely low with only a few hundred calves sold during the 5 years. There is also a small group selling between 20 and 30%.

In all probability there have been no marked changes in the proportions since 1944. The calf-sales map, therefore, is a guide to the extent of the rearing areas of Great Britain at the present time.

Percentage males in the under 1-year-old cattle

Another check on the division of the country into intensive dairying and extensive rearing areas is obtained from the percentage males in the 4 June population of cattle under 1 year old for each county. Some of these are given in Table 4.

Table 4. *A comparison of some of the intensive dairying counties and the extensive rearing counties based on the percentage males (excluding bulls reared for service) in the under 1-year-old cattle (4 June)*

			Percentage males					% Calf sales
Country	System	Counties	1943	1944	1945	1946	1947	1943–7
Wales:	Rearing	Anglesey	48·1	50·2	50·4	49·5	43·5	23·4
		Radnor	48·6	48·4	47·4	45·8	47·1	6·1
	Dairying	Carmarthen	12·6	13·5	10·6	10·8	9·0	65·6
		Flint	17·3	18·8	13·6	13·0	16·1	37·6
England:	Rearing	Northumberland	41·5	41·8	41·1	39·8	37·7	23·2
		Lincolnshire	49·0	48·2	46·2	46·1	44·6	10·6
	Dairying	Cheshire	11·8	11·3	8·5	7·6	8·0	69·0
		Somerset	18·4	16·8	15·2	15·5	14·7	67·6
Scotland:	Rearing	Aberdeen	—	52·1	—	—	—	4·2
		Berwick	—	47·6	—	—	—	6·0
	Dairying	Lanark	—	4·1	—	—	—	55·7
		Wigtown	—	10·2	—	—	—	48·7

The annual percentages of males in the 'cattle under 1 year old' (4 June) have remained remarkably constant for the above counties, and the table indicates the wide differences between the percentages in the dairying and the rearing counties. These results are in general agreement with the calf-sales data. Both tests can therefore be used to define the intensive dairying and the extensive rearing areas of this country, but these tests fail to show internal differences that may be found within each county.

During recent years the dairying counties have shown a reduction in the proportion of male calves reared, whilst the rearing counties have maintained approximately the same annual level.

In 1946 the population of cattle 'under 1 year old' (4 June) in all the rearing counties as above defined (i.e. selling less than 30% of the annual calf crop and rearing more than 30% males) amounted to nearly 440,000, i.e. about one-quarter of the total for Great Britain.

In the same year two large English counties, Northumberland and Lincolnshire, with a high proportion of arable, each had a population of cattle under 1 year old (4 June) nearly equal to the population in the five pastoral rearing counties of Anglesey, Merioneth, Montgomery, Brecon and Radnor in Wales, viz. nearly 50,000.

It does not seem likely that the existing rearing counties will be able to contribute materially to the contemplated cattle-rearing expansion programme of an extra 500,000, because even a 30% increase would amount to only a quarter of the target. Consequently, the extra rearing must be distributed throughout the country and the intensive dairying counties must take a part in it. The immediate contribution of the upland and marginal areas can be of only minor importance in the expansion programme.

If the target is achieved, the cattle population of Great Britain will increase from 8·6 million to 9·8 million (14% increase), and therefore consideration must be given to the food supply, particularly for the winter, necessary to maintain the extra stock.

The annual calf sales per county

The number of calves sold annually through the collecting centres allocated to each county during the 5 years (1943–7) have been examined and certain interesting features are brought out by these totals.

England

The total annual sales of calves in some of the English counties reach very high figures. Thus, Somerset, sold on an average 96,000 calves per annum, or nearly half a million in 5 years, whilst Cheshire, Lancashire, Staffordshire and Wiltshire sold between 300,000 and 400,000 in 5 years, and the West Riding reached nearly 300,000 during the same period. Again, Derby and Dorset are equal in calf sales with over 200,000 in 5 years. Apart from the low figures for the small counties of Rutland and Huntingdon with only three- and four-figure sales, the other east coast counties have a relatively low output in 5 years. Thus Lincolnshire, a very large county, sold an average of only 6000 each year, whilst the East Riding sold 3000 and Northumberland sold 10,500 anually.

Wales

Carmarthenshire sold 43,000 calves each year on an average and 218,607 in 5 years. This is nearly one-third of the annual output of all the Welsh counties. The three counties of Cardiganshire, Denbighshire and Glamorgan each sold over 70,000 calves in 5 years, whilst Radnorshire has disposed of only 2457 calves to the Ministry of Food during the same period.

Scotland

As previously stated, all the Scottish counties to the north of the Forth/Clyde line have a low annual calf sale and also very low percentage calf sales. Most of them reached only four figures and the others less than 20,000 in 5 years.

In contrast the dairying districts of the south-west have a large annual calf sale; thus Ayrshire, the most important, sold 137,000 in the 5 years, a figure which is slightly below the county of Gloucester in England. Lanark is the only other Scottish county to reach six figures in five years (109,712), whilst Wigtown and Dumfries have over 60,000 to their credit.

Changes in the annual calf sales per county

It is of interest to compare the changes in the annual calf sales for the various counties, and it has been possible to group them in accordance with the following behaviour:

Group 1: Counties which show a regular annual decline in numbers of calves sold from 1943 to 1947.

Group 2: Counties which show a rise from 1943 to 1944 and then a decline to 1947.

Group 3: Counties which show a rise from 1943 to 1945 and then a decline to 1947.

Group 4: Counties which show a rise from 1943 to 1946 and then a decline to 1947.

As already indicated, practically all the counties show a sharp decline in calf sales from 1946 to 1947.

Fig. 2 has been prepared to illustrate the above groupings.

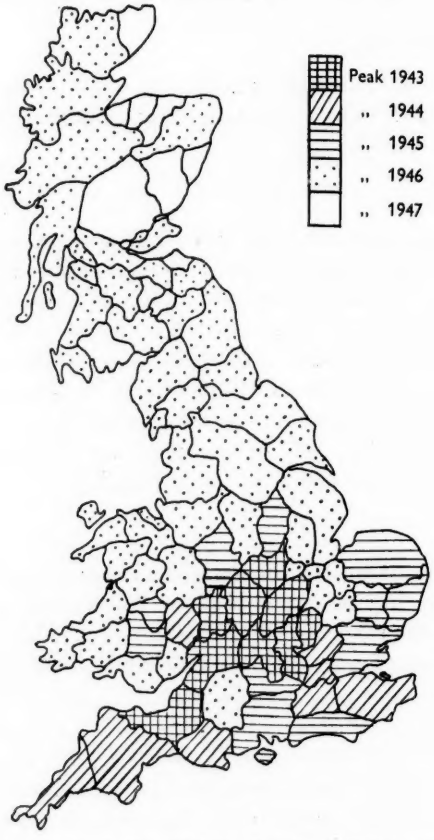


Fig. 2. Peak year of calf sales (1943–7). Counties of England and Wales.

From the map it is seen that counties in group 1 lie together in the south-west midland area from Somerset to Bedford. The map shows that Wiltshire, adjoining group 1, is in group 4, but unlike other areas this county shows only minor changes in its annual calf sales.

Counties in group 2 consist of Cornwall, Devon and Dorset in the south-west, Kent, Surrey, Hertfordshire and Middlesex in the south-east, together with Hereford on the Welsh borders. These counties adjoin group 1.

Counties in group 3 are also adjacent to groups 1 and 2 and consist of the two cattle-rearing counties of Brecon and Radnor in Wales, the three East Anglian counties of Norfolk, Suffolk and Essex, the three southern counties of Hampshire, Berkshire and Sussex, together with the two midland counties of Stafford and Nottingham.

Group 4 consists of the whole of Wales (except Brecon and Radnor); the north of England counties; the east coast counties of Lincoln, Cambridge and Huntingdon, together with practically the whole of Scotland. Wiltshire is the only county in the south-west in this group.

Counties in group 5 are all in Scotland, mainly on the east coast. The annual changes for four of the above groups are illustrated in Fig. 3.

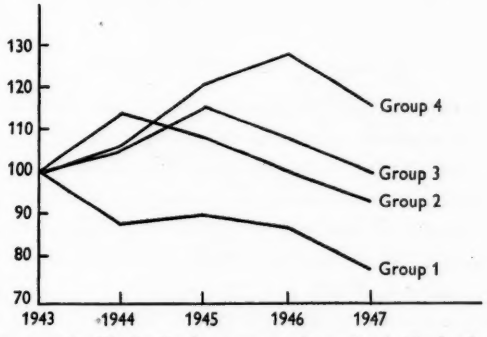


Fig. 3. Comparative annual calf sales for four groups of counties in England and Wales (1943–7).

It is difficult to explain these groupings of counties, as invariably the annual changes in calf sales are apparently unrelated to the amount of the annual rearings. Consequently it is assumed that in group 1 there must be downward changes in the size of the annual calf crop. The other groups show progressive increases in calf sales indicating the continued development of dairying for a varying number of years. It is difficult to say whether the downward trend from the peak year is due to saturation in dairying or to other reasons, such as longer calving intervals and delayed services. The fact that practically all the counties show a downward trend from 1946 to 1947 may suggest that the bad harvesting weather of 1946, together with the severity of the winter 1946–7, actually caused serious casualties among the calves and breeding stock and may have also delayed services and lowered the conception rates. The area covered by groups 1, 2 and 3 lies in the south-eastern half of England, whilst groups 4 and 5 cover the rest of Great Britain. Owing to their importance in milk production, group 1 counties deserve further attention. A comparison of the annual changes that have taken place within this group from 1943 to 1947 is given in Table 5 with 1943 taken as 100 in each case.

Table 5 shows far greater changes in the calf-sales figures than in the others, which have remained remarkably constant throughout. It is at present impossible to account for the peculiarity of group 1, but there may be a common influence acting throughout this important dairying area in such a way as to produce a regular decline in the sales of the

surplus calves and a slight decline in the estimated calf crop. Practically no changes have occurred in the 4 June population of 'cattle under 1 year old' and of the dairy and breeding herds during the period 1943–7. However, for the 3 years 1945–7 for which records of calf births are available, there was a rise from 1945 to 1946 and a sharp drop from 1946 to 1947.

Table 5. *The annual changes in certain calf and cattle numbers from* 1943 *to* 1947 *in Group* 1 *counties* (1943 *as* 100 *in each case*)

Year	Sales of calves to Ministry of Food	The estimated calf crop	4 June population of cattle under 1 year old	4 June population of the dairy and breeding herd	Calf births
1943	100·0	100·0	100·0	100·0	—
1944	88·3	97·0	102·3	99·0	—
1945	90·0	96·5	100·0	97·5	100·0
1946	86·6	97·0	104·5	100·5	100·5
1947	78·6	93·5	102·3	98·0	94·0

Seasonality of the calf sales

In previous publications (Phillips & Davies (1), Phillips *et al.* (2) and Phillips (3)), it has been shown that there is a considerable variation in the seasonality of the calf sales of different areas in the two peninsulas of west Wales and north Wales and also in the seasonal calvings of first-calf heifers. Phillips (3) has also shown the varying seasonal rates of calf births in different parts of the country. It has been postulated that climatic conditions, particularly the average daily hours of sunshine, may have some influence on the development of oestrus during the period December to March and consequently on the rates of the subsequent autumn calvings. It was also shown in these papers that the trends in the calf-sales data were similar to the seasonal trends in milk sales, particularly for the autumn and early winter months. It has, therefore, been suggested that in dairying districts the seasonality of calf sales provides a fairly accurate picture of the seasonality of calvings. The seasonal trends in calf sales are always in the same direction as those of calvings in milk-recorded herds (National Milk Records, M.M.B.), and they are also in agreement with results obtained from the Milk Marketing Board A.I. Centres for 1947–8. Where the calf sales for each collecting centre reach an annual figure of over a thousand, their seasonality provides a fairly accurate reflexion of the seasonality of calvings on the dairy farms in the parishes from which the centre draws its supplies.

In this discussion the average calf sales for the 5 years 1943–7 have been computed on a monthly basis. As in previous publications the year has been divided into three 4-monthly periods as follows: (i) January to April, (ii) May to August, (iii) September to December, and the average percentage calf sales for each of these periods has been calculated. Particular reference has been made to the autumn period (September to December), because it is considered to be the critical period of the year. Calving in this period, of course, implies a conception period of December to March, which was shown by Hammond (12) and Laing (13) to be difficult, partly for the reason that the duration of oestrus is short, and the oestrous cycle is longer at this time of the year. It may also be a period when cows and heifers exhibit anoestrus.

Fig. 4 shows the general picture of the results in which counties with similar autumn-calving percentages have been grouped together.

The map illustrates the general pattern of the average percentage autumn calf sales in this country. Some groups of counties have a high rate of autumn calf sales and others a low rate.

Milk production, and particularly winter production, has been stimulated in this country during recent years. In order to produce higher proportions of winter milk, it is essential for a higher proportion of cows and heifers in the herds to calve during the autumn and early winter months. Many investigators have shown that the average annual milk yield of cows calving in the autumn months is at a higher level (by as much as 20% in some instances) than that of cows calving in the spring, and in many herds the average annual

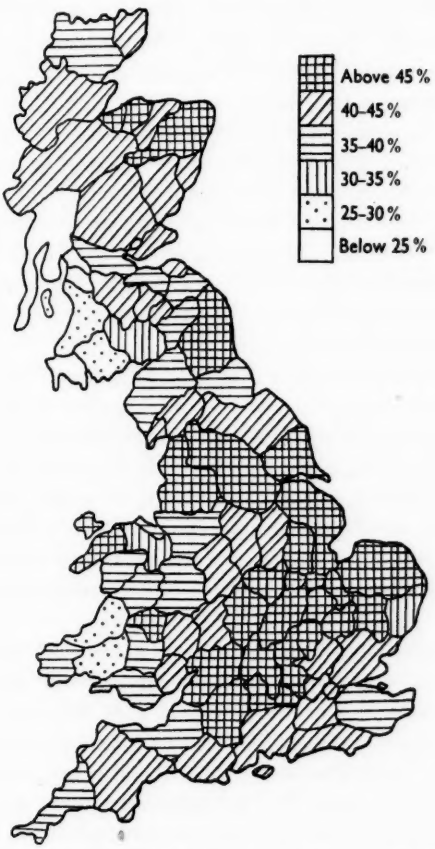


Fig. 4. Average percentage autumn calf sales (1943–7). Counties of England and Wales.

yields of autumn calvers is 200 gal. more than that of the spring and summer calvers. It is, of course, important to provide satisfactory quantities of food for the autumn calvers for efficient winter milk production. In contrast, it may be uneconomic to feed large amounts of concentrates to spring calvers for winter milk production. Producers in general have attempted to obtain as high a proportion of autumn calvers in the herds as possible. The success of the effort varies from district to district, as shown in Fig. 4.

It will be seen from the map that the main area, with over 45% autumn calf sales, appears as a horse-shoe laid across England, stretching from the mouth of the Severn to the Wash and to the Humber, rounding back through the West Riding and Lancashire and stretching across to Anglesey and Caernarvon in Wales. In most of this area of twenty-one counties, the percentage of autumn calf sales is high and the December and January milk sales are about 50% above those of the previous September (cf. Phillips *et al.* (2)). These records apply to the autumn calf sales only, and their significance is not the same in rearing as in dairying districts. For example, the east coast counties of Lincoln, East Riding, etc., have a low autumn rate of calf births (Phillips (3)) but a high rate of autumn calf sales, signifying the differences between rearing and dairying areas. The rates of autumn calf sales are certainly indicative of the possibilities of high autumn calving rates in these areas.

It is of interest to note that there is a tendency for the autumn calf sales percentages to increase from the west to the east coast. Thus, in the north there is a graduation from Lancashire (45·5%), through the West Riding (47·0%) to the East Riding (53·3%), whilst in the south, Gloucestershire (48·5%) and Wiltshire (47·8%) are lower than Norfolk (52·6%) and Suffolk (49·7%) farther east. This west to east cline is true for other areas in England and Scotland, although it is not true everywhere.

The group of counties with a rate of 40–45% autumn calf sales (Fig. 4) lies as a reversed horseshoe embracing the southern wing of the above 45% pattern. The map also shows the region of 35–40% autumn calf sales, and these consist of Cornwall, several counties in Wales, along with Shropshire and Cheshire on the Welsh border, Cumberland in the north-east and the counties of Kent and Durham.

It is, however, the counties with less than 35% autumn calf sales that are interesting, and these are also important dairying counties, or are rapidly becoming important dairying counties. These consist of Cardigan (25·7%), Carmarthen (24·3%), and Denbigh (33·0%) in Wales, together with a group of counties in south-west Scotland consisting of Wigtown (16·8%), Kirkcudbright (25·2%), Dumfries (33·9%), Ayr (29·2%) and Argyle (13·7%). The total sales in the 5 years for all these counties amount to 721,535 calves or 10·5% of the total calf sales of Great Britain during the same period. This total is roughly divided between the three Welsh counties and the five Scottish counties with the advantage in favour of Wales.

The percentage autumn calf sales in Scotland show a definite increase from west to east, and the Wigtown peninsula certainly behaves in the opposite manner to the west Wales and north Wales peninsulas, where the increase is quite definitely from east to west. The general trend in both England and Scotland is an increase eastwards, although it is much more pronounced in the latter than in the former, and is contrary to the trend in Wales. Some confirmation of this eastward cline in England is obtained from the M.M.B. report of A.I. Centres (1947–8) and from the Bureau of Records (M.M.B.) 1946–7 and 1947–8, but in Scotland, in the area to the north of the Clyde/Forth line, relatively few calves were sold, with the result that the trends shown in the map may not be significant.

The above figures conform to the well-known fact that autumn calvers produce a higher average yield than calvers of any other season. The expectation of higher yields with higher rates of autumn calvings is thus borne out. This is true, not only for individual cows in the herds, but also for wide expanses of country, and it is therefore suggested that there must be a common factor at work causing variations in the winter conception rates in this country.

Trends in autumn calf sales (per collecting centre)

The general trends in autumn calf sales are shown in Fig. 5, which has been drawn from the percentages for each collecting centre without reference to county boundaries. The regions with varying rates are shown to fit in with the previous maps, but this map brings out the areas of relatively low autumn calf sales within the various counties which the county map failed to establish. These areas of low autumn calving rates and consequently of high spring calving rates coincide with the farm cheese-making systems of the days gone by where, of course, spring calving was traditionally necessary; for example, an area in Somerset, another on the boundary of Salop, Flint and Cheshire, and the south-west of Scotland. The other remaining area of low autumn calving and of correspondingly high spring calving, viz. Cardiganshire and Carmarthenshire, is less well known for cheese-making, although butter-making, cheese-making and store-cattle rearing was the main system in many parts of this area 50 years ago.

The map, however, brings out the gradualness of the change in space, which suggests that it is not husbandry methods alone that are responsible for the differential calving rates but that other factors, such as climate, play an important part. It is thus apparent that it is fairly easy to obtain a high rate of autumn calvings in some dairying areas but that this is difficult in other areas. Although the system of premiums for winter milk production may be the best arrangement for the country as a whole, it should be realized that such a system operates unfairly when there are some areas which are in the happy position of finding it easy to obtain a high winter production from autumn calvers, whilst this is difficult in other areas.

Discussion

It would appear that to some extent the variations in the rate of autumn calvings are in accordance with the traditional farming systems of the different areas. Thus the well-known rearing areas have a high rate of calf births in spring, and this also holds for the traditional farm cheese-making areas. The autumn calf sales, however, can reflect only the autumn calvings in dairying areas, but in rearing areas it is possible to find, as in Lincolnshire, a relatively low rate of autumn calf births with a high rate of autumn calf sales. This shows the possibility, were it necessary, of this county becoming intensively dairying. Some dairying counties have a high rate of autumn calvings (cf. Caernarvon, Norfolk, Buckinghamshire, etc.), whilst others are particularly low in this respect (cf. Carmarthen, Cardigan and the south-west counties of Scotland).

Again high autumn calf births, high autumn calf sales and high average annual yield per cow seem to increase in England and Scotland from west to east and to coincide with increasing proportions of land devoted to arable farming. The grass-growing west country shows lower values by the above three tests than the arable area of the east. The crux of the whole problem appears to be in the proportion of cows and heifers which become in-calf during the winter months December to March. It is very unlikely that arable crops are better for a high rate of conception in winter than grass and hay or silage and purchased concentrates. Consequently the answer must be found in factors which influence winter oestrus and winter conception rates, and the satisfactory one is that involving climatic variations. The influence of climate directly on the animal and/or indirectly through the food must of course be partly responsible for the variations between different areas. It is further studies on the relationships between climatic factors and animal behaviour that

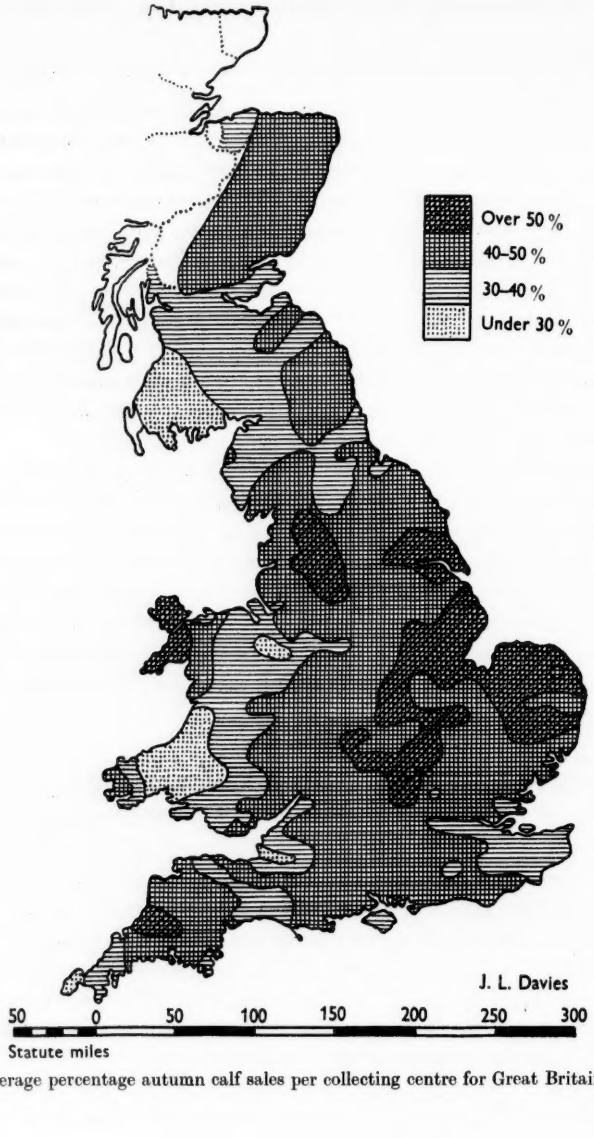


Fig. 5. Average percentage autumn calf sales per collecting centre for Great Britain (1943–7).

will explain the differences and it is already obvious that experimentation on certain aspects of this problem is possible with cattle.

It is realized that the average yield per recorded cow for each county might be influenced by factors other than the seasonality of the calvings. For example, the influence of breeds, the varying proportions in different counties of different breeds from high-yielding ones to low-yielding dual purpose breeds, might be responsible. On the other hand, the varying proportions of the high-yielding breeds is a reflexion of the conditions of soil, fertility and management, both of which are determined to some extent by climatic conditions.

Climatic considerations

Maps (Figs. 4, 5) showing the average percentage of autumn (September to December) calf sales for the 5 years 1943–7 (autumn calf sales by counties and autumn calf sales by Ministry of Food Collecting Centres) reveal several interesting trends.

First taking the country as a whole there is a gradual increase in the percentage of calves sold during the autumn months from west to east. There is a range of approximately 10% in southern England, whereas in Scotland this has increased to the order of 15%. However, in contrast to this general east to west decrease there is the opposite cline in the west Wales peninsula and in north Wales. This reversal in trend is well shown in the more detailed map (Fig. 5) of calf sales per collecting centre.

It has been suggested (Phillips & Davies (1), Phillips *et al.* (2)) that this east to west cline is due in part to climatic conditions within these two peninsulas in Wales. The climatic changes due to the increase in altitude inland are quite marked in their effect. Elsewhere, particularly in central England, such marked changes in climate do not occur because of the absence of high land. The west to east decline in percentage calf sales of these two peninsulas is turned into a much less rapid decline in the opposite direction over England. It would seem that over the greater part of the country there is not the direct climatic effect found in west and north Wales. This reversal of trends, however, calls for comment.

The general decrease in percentage autumn calf sales in the west broadly speaking corresponds with the increased amount of rainfall in the west. It is difficult to attribute any direct influence to rainfall, but its indirect effect upon the farming economy may point to an explanation of the east to west decrease. Thus the proportion of land devoted to arable crops for winter feed increases from west to east, whilst the difficulties and hazards of harvesting fodder crops increase with higher rainfall in the west, and consequently the quality and condition of the harvested fodders is bound to vary accordingly. Secondary factors in themselves due to differing rainfall amounts may give rise to reactions in the animal or in its food, which facilitate or hinder the development of winter oestrus and therefore of autumn calvings. It is possible that where rainfall is high the development of winter oestrus is more difficult, and winter conceptions more chancy than where the rainfall is somewhat less. High rainfall implies a high proportion of overcast skies, reduced intensity of light and fewer hours of sunshine, all of which may affect the animals either directly or indirectly. Again the leaching effects of heavy rains on soil ingredients is well known, and consequently the crops and particularly the harvested winter-feed crops may be deficient in some of the trace substances or in their balance. It has been reported (14) that there is a deficiency of cobalt in the soils in parts of the south-western peninsula and in the Solway peninsula, and both these peninsulas show a reversal of the autumn calving trends shown by the two peninsulas in Wales.

It is true that there are many exceptions to these suggestions, but that merely illustrates the complexity of the picture and of its causes whatever they may be.

SUMMARY

The records of calf sales to the Ministry of Food have been examined for the years 1943–7 and maps made to show variations in (*a*) the percentage disposal of the annual calf crop, and (*b*) the percentage autumn calf sales, in different parts of Great Britain. These have enabled the intensive dairying areas and the extensive rearing areas to be defined.

It is postulated that the variations in the seasonality of calf sales in different areas may be influenced by factors other than husbandry, and that climate may have both direct and indirect effects upon the development of winter oestrus in cattle.

This investigation has formed part of the survey of animal health and production in west Wales and thanks are due to the scientific branch of Messrs Cooper, McDougall and Robertson, Berkhamsted, for financial support. Grateful acknowledgement is made also to the Ministry of Food (Meat and Livestock Branch) and to Major John Francis F.S.I., Carmarthen, for permission to examine the records; to Prof. E. G. Bowen, University College of Wales, Aberystwyth, for helpful criticism; to Messrs R. Jones, Ll. R. Evans and R. D. Williams for their work in collating the material.

REFERENCES

(1) Phillips, R. & Davies J. L. (1949). *J. Dairy Res.* **16**, 1–13.
(2) Phillips, R., Davies, J. L. & Brown, E. H. (1949). In preparation.
(3) Phillips, R. (1949). *Brit. Vet. J.* **105**, 351.
(4) Wood, T. B. & Newman, L. F. (1928). *Beef Production in Great Britain.* Liverpool: R. Silcock and Sons.
(5) Sandars, H. G. (1927, 1928). *J. agric. Sci.* **17**, 339, 502; **18**, 46, 209.
(6) Roberts, E. J. (1929). *Welsh J. Agric.* **5**, 67.
(7) Roberts, E. J. (1926). *J. agric. Sci.* **16**, 416.
(8) Pryse-Howell, J. (1945). *Welsh J. Agric.* **18**, 125–30.
(9) Pettit, G. H. N. (1940). *J. agric. Sci.* **30**, 485.
(10) Marley, J. G. (1947). *J. R. statist. Soc.* **110**, 187.
(11) Smith, J. H. (1946). *Farm Econ.* **5** (6).
(12) Hammond, J. (1927). *The Physiology of Reproduction in the Cow.* Cambridge University Press.
(13) Laing, J. A. (1945). *J. agric. Sci.* **35**, 25.
(14) Corner, H. H. & Smith, A. M. (1938). *Biochem. J.* **32**, 1800.

(*MS. received for publication* 16 *June* 1949)

637.125

393. EXPERIMENTS ON MILKING TECHNIQUE

5. EFFECT OF TEMPORARY CHANGES IN THE INTERVAL BETWEEN WASHING AND MILKING

6. COMPARISON OF ESTABLISHED WASHING AND MILKING ROUTINES

By F. H. DODD, A. S. FOOT and ETTIE HENRIQUES
National Institute for Research in Dairying, The University of Reading

(With 6 Figures)

REVIEW

During the last 3 or 4 years widespread interest has been shown in machine-milking methods, particularly in the technique of stimulating the cow to release her milk immediately before attaching the teat cups. Many dairy farmers believe that the stimulation of the cow to let down her milk immediately before milking, not only reduces milking time, but also increases yield. Reliable experimental evidence of this is, however, still scanty. One difficulty in testing the effect of a method of preparing a cow for milking is that when a new method is introduced experimentally the change itself may affect speed of milking and yields; this may mask the inherent effect of the new routine on milking efficiency.

Miller & Petersen (1) showed that cows which were accustomed to udder washing with hot water immediately before milking dropped in milk and fat yield when the udder washing was done 20 min. before milking and the strip cup used 15 min. before milking. Korkman (2), comparing various methods of preparation for milking, found a significantly slower flow of milk when the interval between udder washing and milking was 10 min. as opposed to 2 min. Ward & Smith (3) compared the yield of the two halves of udders milked at varying intervals after stimulation of 'let-down' by washing the udder with hot water. They found a reduction in milk and fat yield where the half udder was milked 12, 16 or 20 min. after stimulation, but could find no reduction in yield where the period was 4 or 8 min. compared in all cases with the control interval of 2 min.

Very few attempts appear to have been made to compare the effect of established washing routines on milk yield and rate of milking. Dodd & Foot (4), studying cows used to udder washing more than 15 min. before milking, were unable to demonstrate an increase in yield after conditioning them for several weeks to a routine involving a hot-water udder wash immediately before milking. Apart from an increase in rate of milking during the first minute after the teat cups were put on, little change in milking speed was observed. Comparing routines over 5–10 week periods, Knodt *et al.* (5) also failed to demonstrate significant differences in yield where the intervals between washing and milking were varied from 2 to 20 min.

It appears that differences in milking rate due to changes in methods of preparation for milking are mainly the result of altering the time of the 'let-down' of milk rather than of an over-all decrease or increase in rate of flow. This was well illustrated by Smith & Petersen (6), who showed that when cows are stimulated by a hot-water udder wash 2 min. before

putting on the teat cups and the fore-milk withdrawn, a rapid flow is obtained almost immediately the teat cups are put on the cow. On the other hand, where no udder washing or fore-milking was adopted, there was a lag of from 30 to 60 sec. before 'let-down' took place, and although the subsequent rate of flow was similar to the stimulated milking the over-all milking time was increased by the lag.

In the two experiments reported below, further information on the effect of udder washing and the interval between washing and milking has been collected, and by using methods designed to measure very small differences in milking efficiency, two established routines are compared.

EXPERIMENTAL

Experiment 5

In this study the experimental treatments were imposed at single morning milkings on cows accustomed to an udder wash with hot water immediately before milking. Six Shorthorn cows in declining lactation were used; they were milked with a standard bucket-type milking machine fitted with moulded rubber liners and operated at 14 in. mercury negative pressure and at 46 pulsations per minute. The experiment lasted for 13 days, and the experimental milkings were on alternate morning milkings as shown in the experimental plan (Table 1). No attempt was made to force the cows to milk more quickly. At all morning milkings the milkers were asked to begin machine stripping when the rate of machine milking fell below 0·3 lb. in ½ min., and they removed the teat cups when they considered that no more milk was left in the udder and teat cisterns. During the afternoons the duration of milking was at the milker's discretion.

Table 1. *Experimental plan*

	Treatments												
Days	1	2	3	4	5	6	7	8	9	10	11	12	13
Afternoon milkings	All on control treatment												
Morning milkings	C	N	C	N	C	3	C	3	C	6	C	6	C

The control or C treatment was similar to the routine to which they had become accustomed, and for the duration of the experiment the interval between washing and milking was 45 sec. When the cows were on treatment N they were not washed nor given any warning that milking was imminent until the teat cups were put on. Treatments 3 and 6 allowed an interval of 3 and 6 min. respectively between the end of udder washing and the beginning of milking.

The records taken during the experiments were rate of milking readings, as in previous experiments (4), at each morning milking and the weight of milk and fat at every milking.

RESULTS

The effect of the treatments on milking rate is illustrated in Fig. 1, where the mean quantity/time graphs for each treatment are plotted for the first 4 min. of milking. Because of the downward trend in the total morning milk yield three control graphs have been drawn; the control graph in each case being a mean of the three control days next to the treatment days (e.g. in the case of treatment N the control curve is a mean of the readings of the 1st, 3rd and 5th days). The results of a simple statistical analysis of the

treatment differences illustrated in Fig. 2 are shown in Table 2. Where there was no trend in the control values, the analysis was carried out on the differences between treatment means and the mean of all the control values. Where there was a trend the differences between treatment means and the regression of the control readings was analysed. From

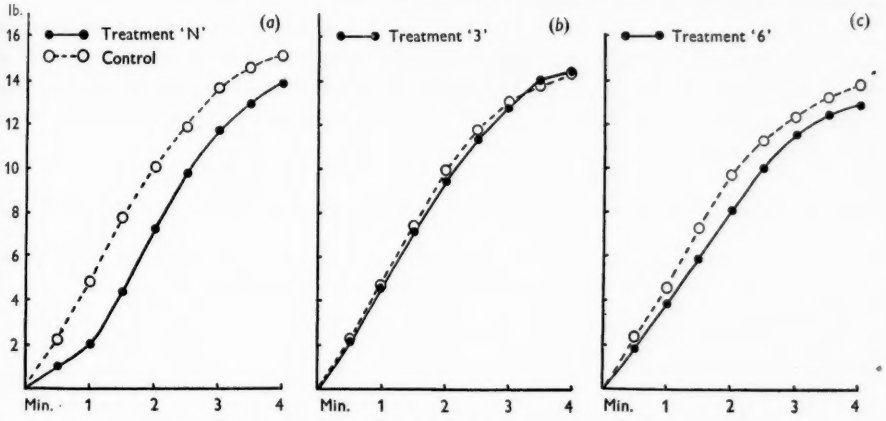


Fig. 1. Quantity/time graphs showing the effect of change in milking routine on rate of milking (Exp. 5).

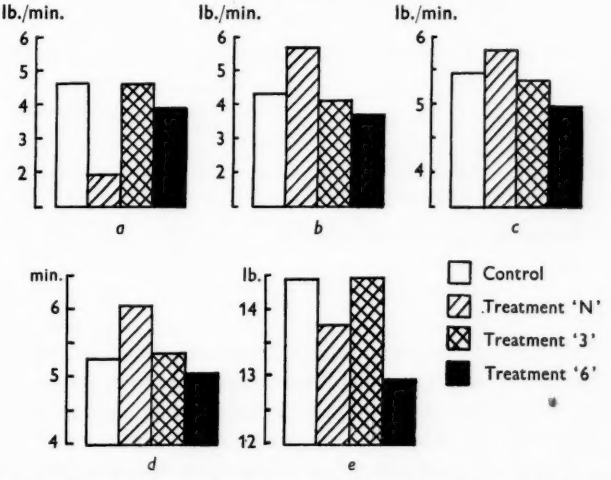


Fig. 2. The effect of changes in milking routine on rate of milking (Exp. 5). (*a*) Milk drawn in first minute. (*b*) Rate of milking in second and third minute. (*c*) Peak flow. (*d*) Duration of milking. (*e*) Milk drawn in four minutes.

Figs. 1 and 2 and Table 2 it is clear that while treatment 3 had no effect, treatments N and 6 had a marked effect on rate of milking. The response to treatment N (Fig. 1*a*) confirms the results of Smith & Petersen (6), who found that there was a delay in the start of milking of up to 1 min. when cows were not stimulated in any way before milking. Reference to Table 2 shows that the reductions in the milk obtained in the first minute and after 4 min. and the increase in the duration of milking were highly significant. The highly

significant increase in the rate of milking in the second and third minute is explained by the fact that when on treatment N the cows reached their maximum rate of milking during this period, whereas on control treatment the rate of milking was rapidly declining by the end of the third minute. The reason for the significant increase in peak flow (the maximum amount of milk in any one minute) is not clear.

The effect of treatment 6 indicates that milking rate was reduced throughout the entire operation. Analysis of the data shows that all the reductions were significant except the rate of milking in the second and third minute of milking. The total duration of milking was not increased by the reduced rate of milking because of a simultaneous reduction in total morning milk yield.

Table 2. *Results of the statistical analysis of the milk yield and milking rate data in Exp.* 5

Treatment	N	3	6	Standard error
Differences between treatment means and control mean				
Milk drawn in 1st min.	−2·70*	−0·01	−0·72†	±0·312
Rate of milking in 2nd and 3rd min.	+0·70*	−0·07	−0·26	±0·194
'Peak flow'	+0·35†	−0·21	−0·47*	±0·126
Total duration of milking	+0·78*	+0·03	−0·26	±0·146
A.M. fat yield − P.M. fat yield	−0·047*	−0·064*	−0·202*	±0·016
Differences between treatment means and the regression line of control values				
Milk after 4 min.	−1·54*	+0·07	−0·79*	±0·207
Total morning yield	−0·46†	+0·09	−0·75*	±0·184

* Significant at 1% level. † Significant at 5% level.

Table 3. *Results of statistical analysis of Exp.* 6

	Unadjusted treatment differences	Standard error
Mean daily yield of milk	+0·597	±0·404
Mean daily yield of fat	+0·198	±0·193
Yield of fat in residual milk	−0·121*	±0·039
Milk drawn in 1st min.	+0·460	±0·212
Rate of milking in 2nd and 3rd min.	+0·831	±0·626
'Peak flow'	+0·237	±0·146

* Significant at 1% level.

The effect of the three treatments on production can be studied in two ways. Apart from the direct comparison of morning yields, it is also possible to study the relative morning and subsequent evening yield on any day that an experimental treatment was imposed and compare these with the relative morning and evening yields on control days. In this way an increase in the amount of milk left in the udder in the morning is emphasized, since the amount obtained at the subsequent evening milking (control routine) tends to increase. The comparison becomes even more revealing when fat production is considered, owing to the high fat content of residual milk left in the udder.

Fig. 3 shows the mean fat yields for the morning and evening milkings for the six cows when subjected to the control and three experimental treatments. The morning production was normally about 20% greater than the evening production, since milking was at about 7.0 a.m. and 4.0 p.m. A reduction in the fat yields at the experimental morning milking followed by an enhanced fat yield at the subsequent evening milking is revealed in each experimental treatment. Although this was small in the N and 3 treatments it was so

large in the 6 treatment that fat production was greater at the evening milking than at the experimental morning milking in spite of the short day interval between milkings (see Fig. 3).

None of the treatments had any measurable effect on the yield of machine strippings.

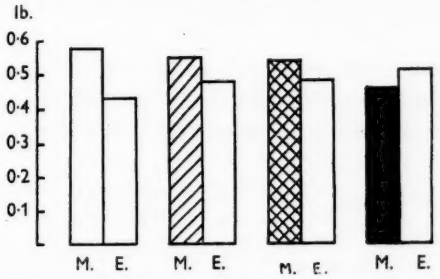


Fig. 3. The effect of change in routine on the mean yield of fat at morning an devening milkings (Exp. 5). M. morning milking.] E. evening milking. For key of treatment see Fig. 2.

Experiment 6

Experiment 6 was designed to compare established routines which differ only in the interval between washing and milking. A comparison of established routines is always difficult in short-term experiments because the effects of treatment are often so small that they tend to be masked by normal day-to-day variations in yield. In order to overcome this difficulty so far as milk and fat yield is concerned a new technique was adopted. At one morning milking each week the residual milk, which remains in the udder after a normal milking, was removed by milking a second time following an intravenous injection of 10 units of oxytocin. The injection was carried out within 15 min. of the end of milking and the second milking followed about four minutes later. The amount of residual milk is inversely proportional to the efficiency of milk removal, and because of its high fat percentage, slight variations in the completeness of milking will be reflected by large changes in the fat content of the residual milk. These changes are much more likely to be significant than changes in normal milk and fat drawn from the udder by a control or experimental milking.

The experiment was carried out with eight cows in early to mid-lactation and lasted 15 weeks. To ensure that, in the first period, the cows were conditioned to their respective routines, each was put on an experimental treatment at calving. This made it necessary to pair the animals on the basis of the milk yield and rate of milking data which had been collected in the previous lactation. Collection of experimental data was not commenced until all animals had reached their maximum daily production. Each group of four animals had one of the following two treatments applied at each milking.

Treatment A: The cows' udders were washed with hot water (100–110° F.), fed their concentrated feeding-stuffs mixture and the foremilk was removed at least 20 min. before milking began.

Treatment B: The preparation for milking was the same as treatment A, except that all three operations were carried out within a minute of the start of milking.

The treatments were reversed after an experimental period of 5 weeks and a further change-over was made after a second period of 6 weeks.

Apart from the measurements of residual milk which were obtained once each week for each cow after a normal morning milking, rate of milking records were taken twice each

week, milk yield was recorded daily, fat on 2 days each week and solids-not-fat estimations on 1 day each week. The milking machine and the method of operation was the same as in Exp. 5, and again no attempt was made to force the cows to milk quickly by early removal of the teat cups.

RESULTS

The effect of the two treatments on the milk and fat yield at the normal milking and the fat yield of the residual milk is given in Fig. 4. All three graphs suggest that treatment B, where preparation was carried out immediately before milking, was the more effective

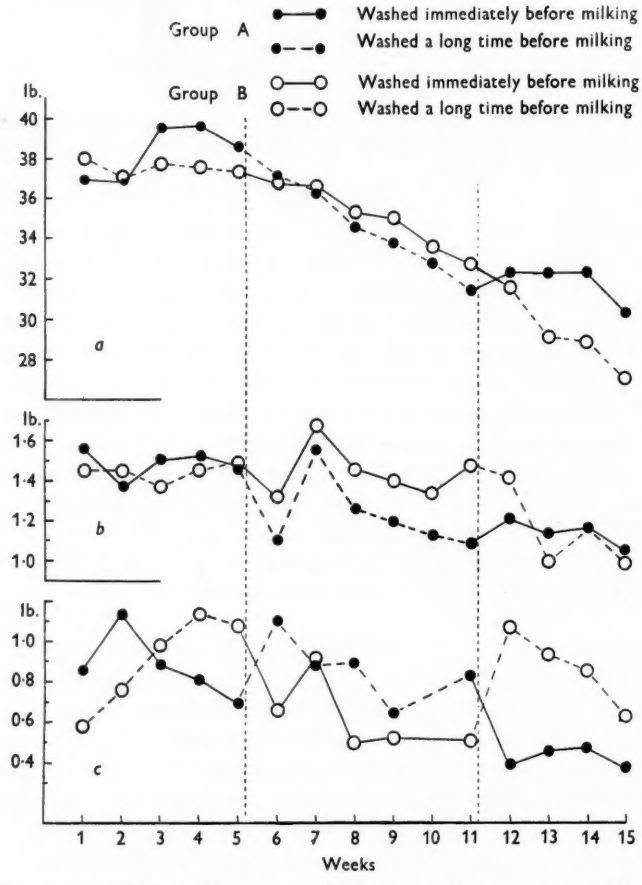


Fig. 4. The effect of two methods of milking on the yield of milk and fat and the yield of fat in the residual milk (Exp. 6). (*a*) Mean weekly milk yield (lb.); (*b*) Mean weekly fat yield (lb.) (*c*) Mean yield of fat in residual milk (lb.).

milking routine. The differences are not significant in the case of milk and fat yield, due probably to the small number of cows used. The effect of the treatment on the fat content of the residual milk is highly significant in spite of the small numbers used.

Fig. 5 shows the effect on rate of milking and illustrates that treatment B was a slightly faster routine. From Table 3 it can be seen that only the effect on amount of milk in the

Group A
Washed immediately before milking
Washed a long time before milking

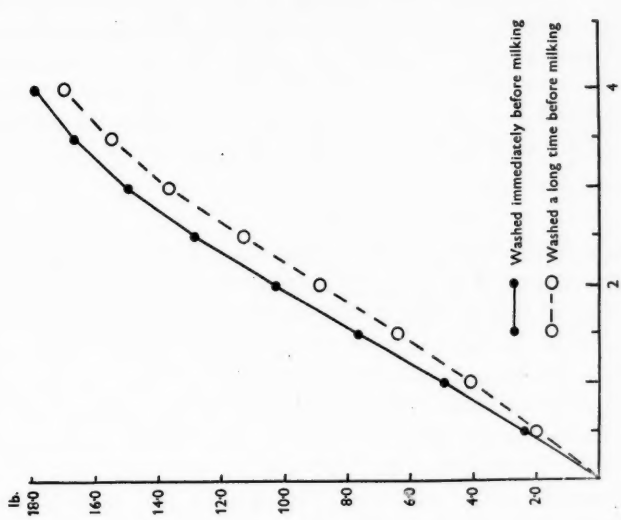


Fig. 6. Mean quantity/time graph showing the effect of two milking routines on rate of milking (Exp. 6).

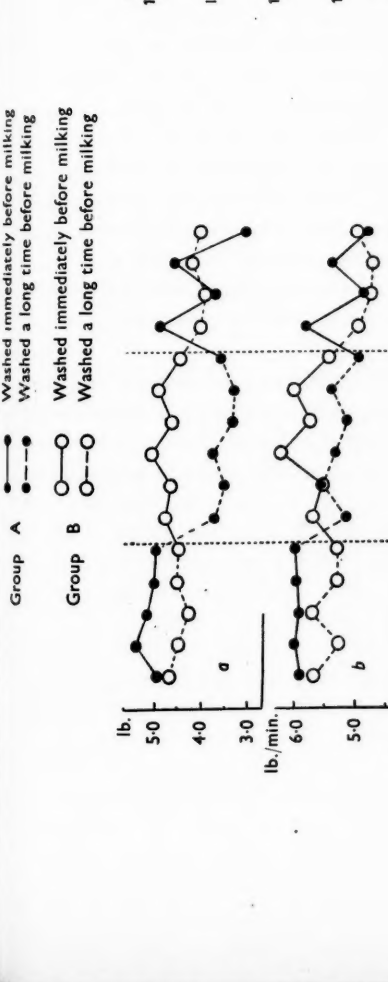


Fig. 5. The effect of two milking routines on rate of milking (Exp. 6). (*a*) Milk drawn in first minute (lb.); (*b*) Peak flow (lb./min.); (*c*) Rate of milking in second and third minute (lb./min.); (*d*) Rate [Total morning yield ÷ total milking time] (lb./min.); (*e*) Total morning milking time (min.)

first minute approaches significance, though the other figures suggest that with more experimental cows the effect on peak flow and rate in the second and third minute would possibly be significant. The mean quantity/time graph plotted for the first 4 min. is given in Fig. 6. This indicates that rate of milking was reduced throughout the whole milking period in much the same way as followed treatment 6 in Exp. 5. Though the rate of milking was greater for treatment B, it was not sufficient to effect a significant reduction in the total milking time, nor was the amount of machine strippings reduced.

CONCLUSIONS

In these experiments we have endeavoured to differentiate between the short-term effect of a change in routine of preparing the cow for milking and the long-term inherent effect of a particular routine. In Exp. 5, where varying methods of preparation were imposed for one milking only on cows used to a particular routine, some marked effects on the 'let-down' of milk and on the efficiency of milking were recorded. Some of these effects were strikingly similar to those reported by Smith & Petersen (6). The inherent long-term effect on production of a method of preparing for milking is often difficult to measure because it may be small compared with the large day-to-day or month-to-month variation in milk and fat production. This applies in particular where the routines tested are within the range of those used in ordinary farm practice. Thus, in a previous experiment (4), in which no control group of cows was available, we failed to demonstrate a difference in production between two preparation routines. In Exp. 6 reported above, where a more precise experimental comparison was made, some significant differences on the subsequent milkings were found where the preparation for milking was carried out less than 1 min. before milking compared with more than 20 min. before milking. Although the difference in favour of the former procedure was not significant as far as milk and fat production is concerned, we believe that a larger experiment would, in fact, show a small advantage for immediate milking compared with milking delayed more than 20 min. The significant increase in residual milk in the latter routine enhances this conclusion, since this would become of considerable importance in early lactation, or at any other stage where udder capacity is limiting production.

Knodt *et al.* (5) could demonstrate no significant difference in yield between routines established for 5–10 weeks where the interval between udder washing and milking varied from 2 to 20 min. On the other hand, the results of Exp. 5, reported above, indicate that where cows are accustomed to a very brief interval between udder washing and milking, extension of this interval to 6 min. at a single milking may have a substantial effect on milking efficiency. In practice, therefore, it appears that it is advantageous to adopt a routine which involves udder washing, fore-milking, and perhaps concentrate feeding immediately before milking, but that once this routine is established care should be taken that the interval between preparation and milking be kept below 2 or 3 min.

From Exp. 6 it appears that a comparison of residual milk yields provides a more precise experimental technique than the normal comparison of milk and fat yields, though this will apply only where experiments are concerned with the efficiency of milk removal as distinct from milk formation.

SUMMARY

1. Cows accustomed to preparation for milking 1 min. before putting on the teat cups were on single mornings (*a*) milked without preparation, (*b*) milked 3 min. after preparation, (*c*) milked 6 min. after preparation.

On treatment (*a*) 'let-down' was delayed but thereafter milking rate was not abnormal. Treatment (*b*) had no pronounced effect on milking efficiency, but treatment (*c*) resulted in a slightly slower rate of milking and a reduced yield of milk and butterfat.

2. In a second experiment a comparison was made between two established routines, in one of which udder washing, fore-milking and concentrate feeding was done less than 1 min. before milking and in the other more than 20 min. before milking. The data suggest that milking immediately after preparation was the more efficient routine, although the differences were not very great.

Our thanks are due to Dr S. J. Rowland, who carried out the tests for the chemical quality of the milk.

REFERENCES

(1) Miller, K. & Petersen, W. E. (1941). *J. Dairy Sci.* **24**, 225.
(2) Korkman, N. (1948). *K. Lantbr. Akad Tidskr.* **87**, 161.
(3) Ward, G. M. & Smith, V. R. (1948). *J. Dairy Sci.* **31**, 694.
(4) Dodd, F. H. & Foot, A. S. (1947). *J. Dairy Res.* **15**, 1.
(5) Knodt, C. B. *et al.* (1947). *J. Dairy Sci.* **30**, 580.
(6) Smith, V. R. & Petersen, W. E. (1948). *J. Dairy Sci.* **31**, 589.

(*MS. received for publication* 11 *June* 1949)

637.127.6:576.8

394. THE INFLUENCE OF TIME AND TEMPERATURE OF STORAGE ON DYE-REDUCTION TESTS IN MILK

1. REDUCTION OF METHYLENE BLUE

By R. K. MORTON and J. M. VINCENT
Milk Research Laboratory, School of Agriculture, University of Sydney

With Statistical Appendix by D. B. Duncan
School of Agriculture, University of Sydney

(With 1 Plate and 3 Text-figures)

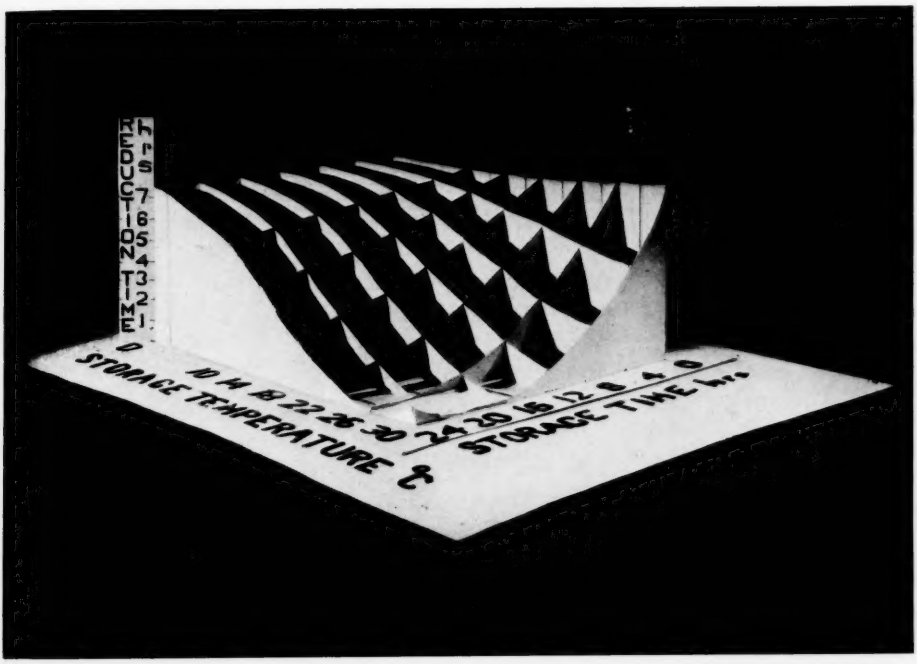
In attempting to apply data obtained in other countries to the determination of suitable dye-reduction standards for the grading of raw milk in New South Wales, it soon became apparent that in order to assess cleanliness of production, the influence of time and temperature of pre-testing storage would have to be investigated(1). A similar need under British conditions has been noted(2), but apart from some data by Frayer(3) for the United States, Johns(4) in Canada, Braz & Hoy(5) and Powell and others(6) for Britain, there is surprisingly little overseas information on this aspect of dye-reduction tests. Smythe(7) has, however, made a very useful contribution which is of particular interest in our case, in that it includes temperatures likely to be encountered in Australia.

It has been our purpose to cover such a range of temperatures as will bridge the gap between Australian and British experience, to extend the conditions dealt with by Smythe in the direction of longer storage times, and to express the simultaneous influence of time and temperature of storage on dye reduction. It is considered that this constitutes a rational approach to the establishment of a standard for the grading of producers on the basis of cleanliness of production, since it permits the use of a compensation scale to allow for seasonal and regional influences beyond the producer's control. A brief account of this work has been published(8).

EXPERIMENTAL

A limited number of producers within easy reach of Sydney was selected on the basis of reports by field officers of the New South Wales Milk Board so as to include 'good' and 'bad' and very different methods of production. Summer and late autumn seasons were represented. It was necessary to ice the summer samples for the several hours involved in transport to the laboratory, but experience has shown that any icing effect is slight and less serious than the uncertainty that would have resulted from carriage at air temperature. Later experience with 10° C. storage suggests it to be more desirable, although not, of course, as easy to achieve and maintain in practice.

On receipt at the laboratory, 10 ml. portions were pipetted, with the usual aseptic precautions, into $6 \times \frac{5}{8}$ in. test-tubes covered with loose-fitting aluminium caps. Enough tubes were put up at the one time to provide for the requisite combinations of time and



Influence of storage time and temperature on methylene-blue reduction time. Mean regression surface, based on six milks.

temperature of storage along with appropriate controls. The following were tested in all combinations:

Time of storage (hr.): 0, 4, 8, 12, 16, 20 and 24.

Temperature of storage (° C.): 0, 10, 14, 18, 22, 26 and 30.

Dye-tests ('old' and 'modified' methylene blue, and resazurin) were performed in the tubes used for storage, either immediately or after holding at 0° C. until the completion of the longest storage period. This latter method was essential for the proper handling of the large numbers of tubes involved in the earlier and major part of the work, and, as shown by later experience, introduced no serious difficulties into the methylene-blue determinations. Readings were made at 5, 10 and 15 min. after commencement, and subsequently at 15 min. intervals to the time of full reduction. Most readings were done in good diffuse daylight, but where icing had to be avoided some night readings were carried out, first with a blue 'daylight' incandescent globe and later with fluorescent 'daylight' tubes (6500° K. type).

RESULTS

Detailed study of six milks

Table 1 gives reduction times obtained for six representative milks sampled during the summer of 1946–7 and exposed to the several combinations of storage time and temperature. The marked effect of both storage factors is apparent.

In the first consideration of the data it has been convenient (compare Smythe (7)) to examine the trends of methylene-blue reduction time (i) for each temperature as a function of storage time, (ii) for each storage time as a function of temperature. At lower temperatures (10–18° C.) the tendency for reduction times to decrease with storage is adequately represented by a straight line, but at 22–30° C. a quadratic term of opposite sign becomes significant. With shorter storage (4 and 8 hr.) linear and quadratic terms are adequate, but with longer storage (12–24 hr.) a cubic term of opposite sign is required.

It is, however, desirable to be able to take account of the trends associated with both storage factors simultaneously. Data in Table 1 have been used (see Appendix) to obtain such a regression function which graphically appears as a surface (Pl. 1) fitted to the forty-nine time-temperature combinations, having twelve parameters and allowing for quadratic variation with storage time, cubic variation with temperature and interactions between the two. The surface based on the means given in Table 1 is expressed by the following equation:

$$Y = 7{\cdot}2 - 0{\cdot}005t + 0{\cdot}0003t^2 - 0{\cdot}047\,T + 0{\cdot}0190\,tT - 0{\cdot}000439\,t^2T + 0{\cdot}0047\,T^2$$
$$- 0{\cdot}002925\,tT^2 + 0{\cdot}0000395\,t^2T^2 - 0{\cdot}000106\,T^3 + 0{\cdot}0000476\,tT^3 - 0{\cdot}000000050\,t^2T^3,$$

where Y is the predicted reduction time in hours; $7{\cdot}2, -0{\cdot}005, \ldots, -0{\cdot}000000050$ represent values for regression constants $b_0, b_1, \ldots, b_{11}$; t = storage time in hours; T = storage temperature °C.

The corresponding 'contour' graph (Text-fig. 1), constructed by joining points of equal reduction times, has the advantage that approximate predicted reduction times can be read off for any combination of storage time and temperature.

Using the same method to calculate regression surfaces for each milk separately, it is possible to examine the validity of the mean surface as an expression of the behaviour of individual milks. The data for this test are presented as an analysis of variance in Table 2.

Table 1. *Influence of storage time and temperature on methylene-blue reduction time*

Storage		Reduction times (¼ hr. units)							
		Milk no.							
Time (hr.)	Temp. (° C.)	1	2	3	4	5	6	Observed mean	Predicted mean
Zero		31	23	32	35	26	25	28·7	28·7
4	10	30	21	30	34	24	23	27·0	27·4
	14	29	19	27	33	21	23	25·3	25·9
	18	28	17	27	30	21	21	24·0	24·0
	22	24	15	26	29	20	23	22·8	21·8
	26	21	13	23	24	18	23	20·3	19·6
	30	20	9	20	23	14	22	18·0	17·4
8	10	29	19	29	34	23	21	25·8	26·4
	14	29	17	26	32	19	21	24·0	23·3
	18	27	13	23	30	17	15	20·8	19·6
	22	21	8	19	23	10	11	15·3	15·7
	26	16	4	14	15	6	9	10·7	12·0
	30	10	1	10	10	2	8	6·8	8·9
12	10	28	18	28	33	18	21	24·3	25·4
	14	25	15	25	29	17	16	21·2	20·9
	18	20	8	19	22	15	13	16·2	15·8
	22	12	3	11	16	11	8	10·2	10·6
	26	8	3	7	11	1	5	5·8	6·2
	30	5	0·3	3	6	0·7	2	2·8	3·2
16	10	29	18	27	32	21	20	24·5	24·3
	14	23	12	22	28	15	14	19·0	18·8
	18	16	4	14	16	11	11	12·0	12·6
	22	9	0·3	6	9	7	4	5·9	6·8
	26	6	0·3	2	5	1	3	2·9	2·3
	30	3	0	0·7	2	0·7	0·3	1·1	0·1
20	10	28	17	27	30	17	23	23·7	23·2
	14	20	9	20	26	12	15	17·0	16·8
	18	12	3	9	14	8	11	9·5	10·1
	22	6	0·3	4	4	3	3	3·4	4·3
	26	3	0	0·3	3	0·3	0·7	1·2	0·3
	30	0·3	0	0·3	2	0·3	0·7	0·6	−0·2
24	10	27	16	27	29	17	18	22·3	22·1
	14	18	7	16	22	11	15	14·8	15·1
	18	10	1	6	12	6	6	6·8	8·1
	22	4	0·3	2	5	1	3	2·6	2·6
	26	2	0	0	2	0·7	1	1·0	0·0
	30	1	0	0	0·3	0·3	0	0·3	2·2

0 indicates immediate loss of colour; 0·3 white at 5 min.; 0·7 white at 10 min.

Table 2. *Pooled analysis of variance for regression surfaces based on six producers*

Source of variation	Degrees of freedom	Sums of squares	Mean square
1. POOLED SURFACES	71	35,476·658	
1·1. Between means	5	4,176·861	835·372
1·2. Pooled regressions	66	31,299·797	
1·2·1. Average regression	11	30,374·574	2,761·325
1·2·2. Between regressions	55	925·223	16·822
2. POOLED DEVIATIONS FROM SURFACES	222		
2·1. Error	150	342·481	2·283
2·2. Rejected from error*	72		
Total	293	35,819·139	

Variance ratios

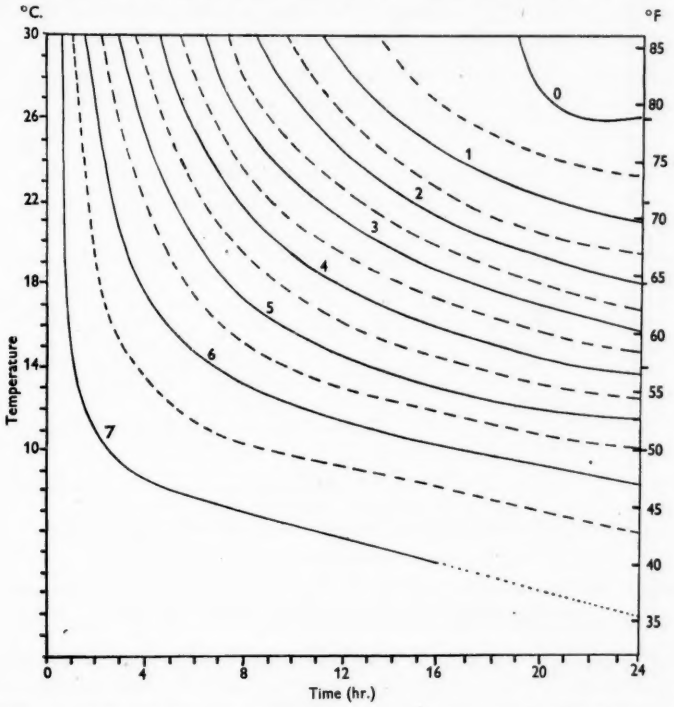
Between means (item 1·1 ÷ item 1·2·2): 49·659, P <0·001.
Residual surfaces (item 1·2·2 ÷ item 2·1): 7·368, P <0·001.

Note *. 72 degrees of freedom are rejected from the pooled deviations when these are used to estimate error. This is done to compensate for the fact that the thirteen reduction values for zero storage time and temperature for any one producer were all given the same observed value. Thus 12 degrees of freedom were lost for each producer.

The entire variation breaks into two parts: item 1: variation accounted for by the surfaces; item 2: error variation of the observed points about the surfaces.

Because of the method of calculating the surfaces (see footnote to the table) sub-item 2·1, with fewer degrees of freedom, has been used as the more conservative estimate of error. The smallness of this error mean square indicates the very good fit to the experimental points provided by the regression functions used to determine the individual surfaces.

Item 1 is divided into two parts, viz. sub-item 1·1, measuring variation between average heights of individual surfaces (i.e. between milk averages) and sub-item 1·2, the remaining variation associated with the surfaces. The average surface (1·2·1) accounts for most of this, although the residual (1·2·2), which measures variation among surface shapes, is highly significant when compared with the small error term (item 2·1).



Text-fig. 1. Influence of storage time and temperature on methylene-blue reduction time. Contour graph of mean regression surface. Lines join points of equal reduction time in hour and half-hour intervals.

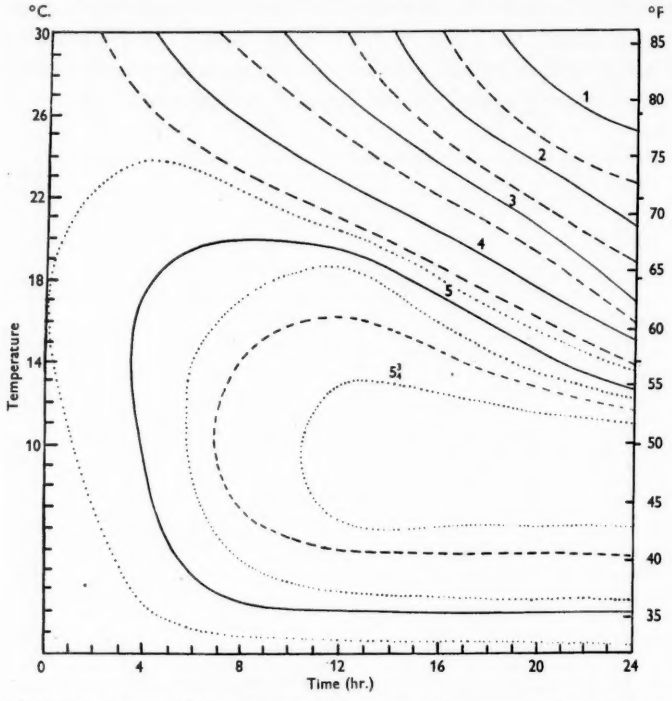
Such differences in surface shape indicate that certain combinations of storage time and temperature are likely to permit greater discrimination between producers. For this purpose the variation between the six surface values at each storage combination, expressed as a standard deviation, has been plotted as a contour graph (Text-fig. 2). A higher standard deviation indicates greater discrimination.

Results with other milks

Samples on a second occasion (autumn 1948) for three producers have been studied in the same way. Again, it has been found that the influence of time and temperature of

storage on reduction time can be well described by a regression function of the same form as the equation already given, most of the variation between producers being accounted for by differences in surface height rather than shape. Mean surfaces obtained on both occasions have been compared in the analysis of Table 3.

Here, too, the appropriate error term (sub-item 2·1) is very small, and the average surface (sub-item 1·2·1) is a very good fit to the observed means. The difference between occasions is principally associated with surface heights (sub-item 1·1), whilst the difference



Text-fig. 2. Relative sensitivity of methylene-blue test after storage. Lines join points of equal standard deviation expressed in quarter-hour units.

Table 3. *Analysis of variance for regression surfaces established on two occasions*

Source of variation	Degrees of freedom	Sums of squares	Mean square
1. POOLED SURFACES	23	9,195·434	
1·1. Between occasion means	1	170·070	170·070
1·2. Pooled regression	22	9,025·364	
1·2·1. Average regression	11	8,967·248	815·204
1·2·2. Between regressions	11	58·116	5·283
2. POOLED DEVIATIONS FROM SURFACES	74		
2·1. Error	55	65·366	1·188
2·2. Rejected from error*	19		
Total	97	9,260·800	

Variance ratios

Between means (item 1·1 ÷item 1·2·2): 32·192, $P < 0·001$.
Residual surfaces (item 1·2·2 ÷item 2·1): 4·447, $P < 0·001$.

Note *. Nineteen degrees of freedom are rejected from the pooled deviations to allow for occasions when zero storage time and temperature were given the same observed reduction time.

between surface shapes (sub-item 1·2·2) is small, though highly significant compared with the small error term (sub-item 2·1).

As noted above the detailed part of the investigation involved icing periods before and after storage treatments. Samples tested immediately on receipt and compared with similar samples held at 0° C. for 24 hr. (representing the extreme case) indicated that any effect of this treatment differed between milks and when operative was small in so far as the methylene-blue test was concerned (compare Wilson and others (10)). Although for a particular producer it might be more serious, the average regression surface would be little affected.

The effect of post-storage icing has been examined in more detail for the three milks studied in autumn, 1948. At this time of the year it was possible to maintain the samples at 10° C. for the transport period of not more than 3½ hr. On arrival at the laboratory, duplicate samples were exposed to the same storage combinations as were used in the earlier part of the work. One group was then tested immediately on completion of storage, the other being iced and set up simultaneously as before. Data are analysed in Table 4.

Table 4. *Analysis of variance for regression surfaces established with and without post-storage icing*

Source of variation	Degrees of freedom	Sums of squares	Mean square
1. POOLED SURFACES	23	7,736·690	
1·1. Between treatment means	1	19·221	19·221
1·2. Pooled regression	22	7,717·469	
1·2·1. Average regression	11	7,662·703	696·609
1·2·2. Between regressions	11	54·766	4·979
2. POOLED DEVIATIONS FROM SURFACES	74		
2·1. Error	62	109·088	1·759
2·2. Rejected from error*	12		
Total	97	7,845·778	

Variance ratios

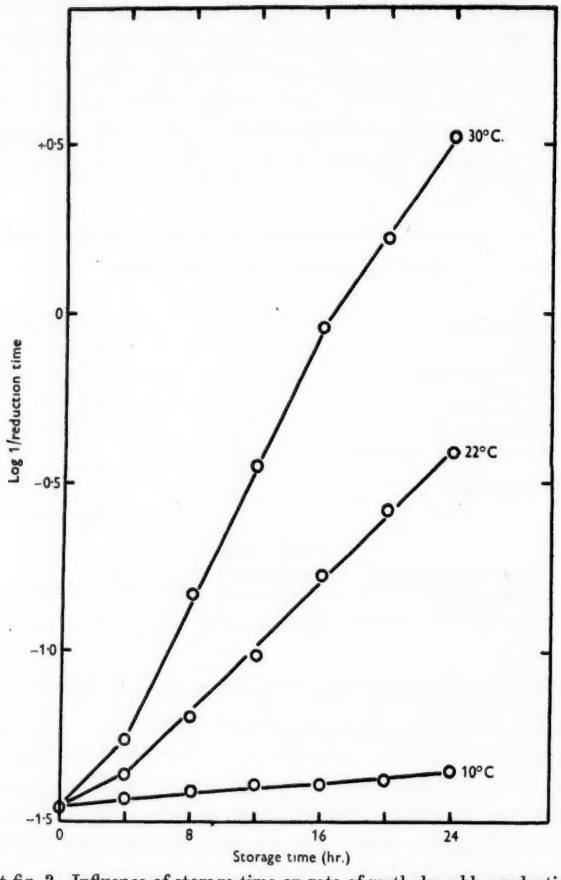
Between means (item 1·1 ÷ item 1·2·2): 3·861, $P > 0·05$.
Between surfaces (item 1·2·2 ÷ item 2·1): 2·830, $0·01 > P > 0·001$.

Note *. Twelve degrees of freedom rejected as for Tables 2 and 3.

The difference between the heights of the two mean surfaces is slight (less than ¼ hr. longer with icing) and is not significant. Some significance attaches to differences in the shape of the two mean surfaces (sub-item 1·2·2), but this is small relative to differences in the surface shapes of different milks (Table 2), and has to be balanced against difficulties in obtaining exactly comparable readings under artificial light as well as the important fatigue factor that becomes serious when the observer attempts to set up tubes and read continuously through the dark as well as the daylight hours. With resazurin tests—to be dealt with in a further report—the effect of icing is more serious and necessitates certain adjustments.

DISCUSSION

Although the samples used in the present investigation were necessarily restricted in number to permit a detailed study of the requisite time-temperature combinations, they were of milks produced under widely different conditions and on different occasions. The relative constancy of shape for the regression surfaces (Tables 2–4) and the goodness of fit of each surface to the experimental points, shown by the small error term (e.g. sub-item 2·1 in Table 2), show that the relationship expressed in the equation describes in a fundamental way the fall in methylene-blue reduction time that results from storage.



Text-fig. 3. Influence of storage time on rate of methylene-blue reduction.

By converting reduction times to rates, and plotting their logarithms against time, it is seen (Text-fig. 3) that the increase in dye-reduction activity with time parallels the curves commonly obtained for the growth of bacteria in a liquid medium. The changes in reduction time resulting from storage thus appear to be explained by the influence of time and temperature of storage on bacterial growth.

The average surface for six producers permits the performance of a milk after a given storage treatment to be predicted with a fair measure of accuracy. The applicability of the

surface expressed in the equation as a general measure of the influence of storage on reduction time is indicated by agreement with data obtained on the later occasion:

Fall in reduction time with storage ($\frac{1}{4}$ hr. units)	Occasion 1	Occasion 2
10° C.; 8 hr.	2·3	2·0
16 hr.	4·4	4·4
18° C.; 8 hr.	9·1	9·6
16 hr.	16·1	16·1
26° C.; 8 hr.	16·8	17·4
16 hr.	26·4	25·6

The results also gain in validity from agreement with other workers. Using the linear values obtained by Smythe (7) in Queensland, the fall in reduction time on 4 hr. storage at several temperatures can be compared with times predicted from our equation:

Storage temperatures (° C.)	Fall in reduction time with 4 hr. storage ($\frac{1}{4}$ hr. units)	
	Present investigation	Smythe's data
10	1·2	−0·3
15	3·3	1·0
20	5·7	4·4
25	8·1	7·2
30	10·6	10·6

It will be observed that the fall for the lower temperatures is rather greater in our case than that recorded by Smythe (at 10° C., the latter found no significant change over the 5 hr. storage period), but the agreement is probably as good as could be expected, particularly in view of the fact that Smythe's milks were exposed to high temperatures for about 2 hr. before starting the storage period. Johns (4) gives reduction times for Canadian milks representing extreme bacteriological quality with and without storage at 13° C. for 18 hr. Exposure to such storage lessened reduction time by 11·2 quarter-hours, compared with 10·0 quarter-hours expected from our results. Braz & Hoy (5), in Britain, state that a milk stored for 12 hr. at 18·3° C. is equivalent to one for 18 hr. at 15·5° C. Inspection of Text-fig. 1 shows that, in our experience, the reduction times for these two storage combinations are, in fact, the same.

Whilst then it is not to be expected that each milk will conform to exactly the same surface, it is apparent that the mean case described in the equation enables comparison of milks produced in different localities and under different seasonal and production conditions. No more reasonable basis for comparison seems possible.

In order to relate New South Wales production to the British summer standard (11), mean summer data at Kew (12) for the 12 hr. period 6 a.m. to 6 p.m. (morning milks) and the 18 hr. from 4 p.m. to 10 a.m. (evening milks), May to October 1930–7, have been utilized. The mean temperatures thus obtained are 16 and 14° C. As it happens the mean surface (Text-fig. 1) passes through this standard ($4\frac{1}{2}$ hr. reduction time) at both time-temperature combinations and can therefore be used directly to relate performance under varying storage conditions to this standard. In any case the height of the mean can be readily adjusted to meet the requirement of any standard by appropriate change in the b_0 term of the equation.

Furthermore, the mean regression surface, particularly in the form of Text-fig. 1, can

be used to establish a rational storage compensation scale that will largely free the test from undue dependence on atmospheric temperature. This would meet the need expressed by Mattick, Hiscox & Davis (2) when they stated that 'some reform in the management of samples prior to the examination seems to be necessary if the result of the bacteriological examination is in fact to justify the common belief that the conditions of production...are measured by such an examination of the milk'. It is of some interest to note also that, as might be expected from the experience of a number of workers (e.g. Barkworth, Irwin & Mattick (9)), the British winter standard of 5½ hr. is appreciably lower than the summer standard, which according to Text-fig. 1 corresponds with 7 hr. under average British winter conditions (12 hr., 6° C.).

Fundamental studies of the sensitivity of the modified methylene-blue test were made by Barkworth *et al.* (9) in comparing the dye test with the plate count as a means of bacteriological grading of milk. Wilson and others (10) state a need for a storage period before testing, and this principle is accepted in British practice. The studies of these authors did not, however, include an examination of the effects of storage time and temperature on sensitivity. Data obtained in the present investigation have been utilized to determine the storage treatments likely to provide most discrimination between producers. The practical significance of such differences is indicated in the present case, in that as the standard deviation decreases from six to one, the difference in reduction times between the best and worst of the six milks reduces from 16 to ¼ hr. It will be seen that there is an optimal storage range, longer storage being desirable at lower temperatures but restriction of storage is essential at higher temperatures if sensitivity is to be maintained. Text-fig. 2 justifies the use of 12 hr. storage in the British Ministry of Health method, since this gives high sensitivity over the normal range of English temperatures (mean 6° C. in winter, 16° C. in summer, at Kew). Such a storage would be applicable to the New South Wales winter but too long for the summer period (temperatures of the order 22–24° C.) when a storage of 4–8 hr. is more suitable.

In practice it is desirable to maintain milk samples at atmospheric temperature for a period (considered from time of production) likely to give a high level of sensitivity, and such that all producers will have their milk received at the depot within the storage time. By this provision, time between production and receipt at the depot is counted as part of the storage, and the influence of distance from the receiving depot can be minimized so as to permit a better measure of cleanliness of production. The permitted reduction time can then be set according to the air temperature prevailing for the given storage period, so that a comparable standard is maintainable throughout the year. Recommendations at present under test in New South Wales have been based on these principles. Results to hand over a 6-month period (13) show that whilst there would otherwise be a marked dependence on air temperature, the influence of this factor has been largely obviated by the application of the temperature-compensation scale based on Text-fig. 1.

Although the present paper is primarily concerned with methylene-blue reduction by the modified method, data were obtained concurrently with the resazurin and old methylene-blue tests, the latter since it is still in use in this State. In this way it has been possible to compare standards based on different dye tests, and it seems to us that the British resazurin standard (14) is appreciably lower than the methylene blue (11).

A good deal of attention has been given to the nature of resazurin reduction as a function of time and, although time to disk 0, or disk 1, closely parallels methylene-blue reduction,

factors altering the shape of the reduction curve are likely to affect decisions depending on intermediate disk readings. In this connexion it has been found that exposure to low temperatures is likely to have a marked effect. It is hoped that this and other aspects of resazurin tests will be the subject of a further communication. It has been practicable, meanwhile, to use the data so far collected, with reasonable corrections, to formulate interim resazurin standards that are under test alongside recommendations for methylene blue.

SUMMARY

Milks from representative producers have been used to study the influence of storage time and temperature on methylene-blue reduction times. The trends of reduction times are very well expressed as a regression function of both storage factors simultaneously, which therefore appears to describe a fundamental property of raw market milk. It thus becomes possible to compare standards in different countries and producing regions and, by the application of a rational temperature-compensation scale, to maintain reasonably constant production standards throughout the year.

The same data have permitted an examination of the discriminating ability of the dye test as influenced by pre-testing storage. It is apparent that at lower temperatures longer storage increases the test's 'sensitivity', but a shorter storage time is essential when higher temperatures prevail. The storage period can therefore, within certain practical limits, be adjusted to obtain greater sensitivity in the test.

These principles have been applied in the establishment and testing of provisional standards for New South Wales. The present data, however, particularly as expressed in Text-figs. 1 and 2, should permit ready application to other centres.

Data obtained at the same time with other dye tests permit a comparison of the several tests and standards based on them. The resazurin test is being examined in some detail.

The work is part of a co-operative project between the New South Wales Milk Board and the Agricultural Microbiology Section of the School of Agriculture, and the authors are glad to acknowledge the constant interest shown by the Executive and Officers of the Board and the skilled technical assistance rendered by Miss P. McGrath. We are particularly indebted to Dr D. B. Duncan for detailed advice in connexion with statistical aspects.

REFERENCES

(1) MORTON, R. K. & VINCENT, J. M. (1947). *J. Aust. Inst. agric. Sci.* **13**, 125.
(2) MATTICK, A. T. R., HISCOX, E. R. & DAVIS, J. G. (1939). *J. Dairy Res.* **10**, 515.
(3) FRAYER, J. M. (1934). *Bull. Vt. agric. Exp. Sta.* no. 374.
(4) JOHNS, C. K. (1939). *Sci. Agric.* **19**, 435.
(5) BRAZ, M. & HOY, W. A. (1938). *J. Dairy Sci. Abstr.* **21**, 304.
(6) POWELL, H. M., JENKINS, E. & THOMAS, S. B. (1939). *Welsh J. Agric.* **15**, 164.
(7) SMYTHE, V. R. (1945). *Qd. J. agric. Sci.* **2**, 128.
(8) MORTON, R. K. & VINCENT, J. M. (1948). *Nature, Lond.*, **162**, 415.
(9) BARKWORTH, H., IRWIN, J. O. & MATTICK, A. T. R. (1941). *J. Dairy Res.* **12**, 265.
(10) WILSON, G. S. *et al.* (1935). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 206.
(11) GREAT BRITAIN, Ministry of Health Memo. 139/Foods, 1937.
(12) GREAT BRITAIN, Air Ministry, *The Observatories Year Book* (1930–7), M.O. 430.
(13) FEAGEN, J., MORTON, R. K. & VINCENT, J. M. Unpublished data.
(14) GREAT BRITAIN, Ministry Agric. and Fish., Form 158/TPY, 1943.

APPENDIX ON REGRESSION METHODS

By D. B. Duncan

Preliminary examinations showed that (*a*) a set of seven quadratic functions would adequately measure the regression of reduction time Y on storage time t for each of the seven storage temperatures T, and (*b*) a set of seven cubic functions would adequately measure the regression of Y on T for each of the seven values of t. Hence the selection of the function

$$Y=b_0+b_1t+b_2t^2+b_3T+b_4tT+b_5t^2T+b_6T^2+b_7tT^2+b_8t^2T^2+b_9T^3+b_{10}tT^3+b_{11}t^2T^3, \quad \text{(A)}$$

to measure the simultaneous regression of Y on t and T. All of the functions of this form which were used were fitted by the common method of least squares. Any attempt to do this on the combinations of the t and T variables as given would be unwieldy, and the following variables were introduced to replace the latter:

$$\begin{aligned}
z_1 &= \tfrac{1}{4}(t-\bar{t}), & z_2 &= (z_1^2-\overline{z_1^2}), & z_3 &= \tfrac{1}{2}(T-16),\\
z_4 &= z_1z_3, & z_5 &= (z_1^2z_3-\overline{z_1^2z_3}), & z_6 &= z_3^2-\overline{z_3^2},\\
z_7 &= z_1z_3^2, & z_8 &= (z_1^2z_3^2-\overline{z_1^2z_3^2}), & z_9 &= (z_3^3-\overline{z_3^3}),\\
z_{10} &= z_1z_3^3, & z_{11} &= (z_1^2z_3^3-\overline{z_1^2z_3^3})
\end{aligned}$$

The following function similar to A was fitted to the data in each case

$$Y=b_0'+b_1'z_1+b_2'z_2+\ \dots\ +b_{11}'z_{11}. \quad \text{(A')}$$

and the ultimate form A was obtained from this form by replacing the variables by the corresponding combinations of the t and T variables. The z variables were chosen in the above way to be whole numbers as small as possible for all values of t and T. If the T values were evenly spaced it would be possible to choose orthogonal variables instead of the z variables with very little effort and thus reduce much of the work in fitting equations corresponding to A'. However, in the present case, the work involved in getting orthogonal variables would be equivalent to the work it would save later.

Since several equations of the form A' were to be fitted, each having the same set of independent variables, the sums of products matrix $B=[S(z_iz_j)]$

$$\begin{bmatrix}
S(z_1^2), & S(z_1z_2), & \dots & S(z_1z_{11})\\
S(z_1z_2), & S(z_2^2), & \dots & S(z_2z_{11})\\
\vdots & & & \\
S(z_1z_{11}), & S(z_2z_{11}), & \dots & S(z_{11}^2)
\end{bmatrix}, \quad \text{(B)}$$

was first inverted to give the matrix

$$\begin{bmatrix}
C_{11}, & C_{12}, & \dots & C_{11}\\
C_{21}, & C_{22}, & \dots & C_{2\cdot11}\\
\vdots & & & \\
C_{11\cdot1}, & C_{11\cdot2}, & \dots & C_{11\cdot11}
\end{bmatrix}, \quad \text{(C)}$$

which was then used for all the required fittings(1).

The cumbersome work involved in inverting the matrix B was eased somewhat by first transforming it to a matrix B′. The jth element in the ith row of B′ was obtained from the corresponding element in B by dividing the latter by $\sqrt{[S(z_i^2)\ S(z_j^2)]}$. Thus B′ was a matrix of correlation coefficients. This was then inverted to give a matrix C′ and the required matrix C was obtained from C′ by dividing its ijth element by $\sqrt{[S(z_i^2)\ S(z_j^2)]}$. The intercorrelation matrix B′ was easier to invert than the sums of products matrix B because the elements of the latter were very heterogeneous in the significance of corresponding decimal places.

REFERENCE

(1) FISHER, R. A. (1946). *Statistical Methods for Research Workers.* Edinburgh: Oliver and Boyd.

(*MS. received for publication* 3 *February* 1949)

541.132.3:614.484

395. THE INFLUENCE OF ALKALINITY ON THE GERMICIDAL ACTION OF HYPOCHLORITES*

By C. E. CHAPLIN AND C. K. JOHNS

(With 2 Figures)

The germicidal efficiency of hypochlorite solutions is impaired by increasing alkalinity. This has been demonstrated repeatedly in the laboratory (1, 2, 3, 4, 5) and in practical tests (4, 6), and has been explained on the assumption that undissociated hypochlorous acid is the active component whose concentration is a function of the hydrogen-ion concentration (7, 8).

Recently, Cousins & Wolf (9) and Neave & Hoy (10) have presented results of experiments which are not in agreement with the above. According to the former authors pH 9·5 and 11 are the optimum values when using 25 and 200 p.p.m. of chlorine respectively for the destruction of thermoduric micrococci. Similarly, Neave & Hoy claim that solutions at pH 10 and 11 are superior to those at pH 7 in killing *Staphylococcus aureus*. Both groups of workers used diluted milk films dried on metal surfaces.

To examine this work, a series of experiments was undertaken in which *Staph. aureus* was used throughout as a test organism, and 200 p.p.m. available chlorine was maintained as a standard concentration. A neutral and an alkaline hypochlorite solution were compared.

METHODS

The liquid suspension method of evaluating germicides shows that hypochlorite solutions at neutrality are more effective than those at high alkalinity (5, 11). Johns (1) has shown agreement in the disinfection of a moist film on a glass surface when immersed in the disinfectant, but Neave & Hoy introduced modifying factors when they investigated the disinfection of dried films. Drying a bacterial suspension on a surface would logically prolong the killing time but would not necessarily explain the relative loss of activity of the neutral solution unless, as they suggest, rehydration of the film is hastened by the more alkaline solution.

Using Neave & Hoy's tray method we were in agreement with their results, but we felt that the spotted deposit on the surface was unnatural and that the concentration of milk solids was greatly in excess of what might be expected. A test of this showed that when 1 ml. of 10% milk was permitted to flow over the surface of the tray it covered better than 20 times the area covered by the spots prepared according to Neave & Hoy's method from the same amount of diluted milk.

The nature of the surface was considered as a possible modifying influence. Survivors on glass trays were compared with those on tin trays, and survivors on glass slides with those on tin and stainless steel strips of the same dimensions as the slides. The results were essentially the same regardless of the surface.

* Contribution no. 284 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

The various methods used in this type of work are essentially identical and consist in contaminating an arbitrarily chosen object and then disinfecting it by immersion in the germicidal solution under examination. Choice of a method may therefore be made on the basis of simplicity alone. For this reason we decided to continue with Johns's slide method (1), though this was modified to provide greater sensitivity and accuracy. The modification consisted of shaking the treated slide in sterile sodium thiosulphate to remove as many adhering organisms as possible and plating the slide and the thiosulphate solution. Total survivors per slide were calculated from the number of colonies appearing on the plated slides and on the thiosulphate plates.

In this way dried and moist films of 10% and whole milk were treated with hypochlorite solutions at pH 7·2 and 10·5.

RESULTS

Survivors at appropriate time intervals were calculated and adjusted to the rate per 100,000 of the control slides. Log averages of the results are shown in Table 1. These figures represent at least six test slides in the series where good agreement was obtained, and as many as twelve slides with dried whole milk films where irregularities were prone to occur.

Table 1. *Survivors per* (10^5) *of hypochlorite treatment. Log averages*

Nature of film	pH of hypochlorite	Time of treatment (sec.) 2	5	10	20	40
Dried whole milk	10·5	—	4·29	3·82	3·30	2·97
	7·2	—	4·21	3·75	3·36	2·99
Dried 10% milk	10·5	4·49	3·43	2·62	2·05	—
	7·2	3·32	2·63	1·71	1·79	—
Moist whole milk	10·5	4·05	3·21	2·50	2·05	—
	7·2	3·41	2·62	2·33	1·82	—
Moist 10% milk	10·5	3·10	2·11	2·00	—	—
	7·2	2·62	1·76	1·54	—	—

In Fig. 1 survivors in the four types of films are compared for each hypochlorite solution. This figure shows that the whole-milk film dried on the surface is disinfected most slowly,

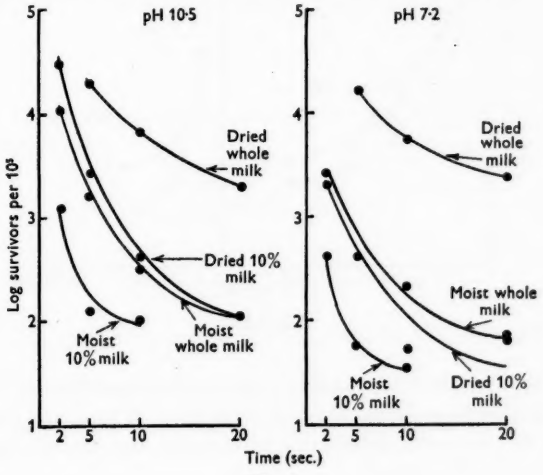


Fig. 1

and of the four treatments the moist 10% milk film was the most easily disinfected. The two other curves lie between these extremes and are nearly equal in value.

Fig. 2 shows that the effect of low pH is increased with a decrease in milk content. The pH effect, absent in the dried whole-milk film, becomes apparent in the moist film.

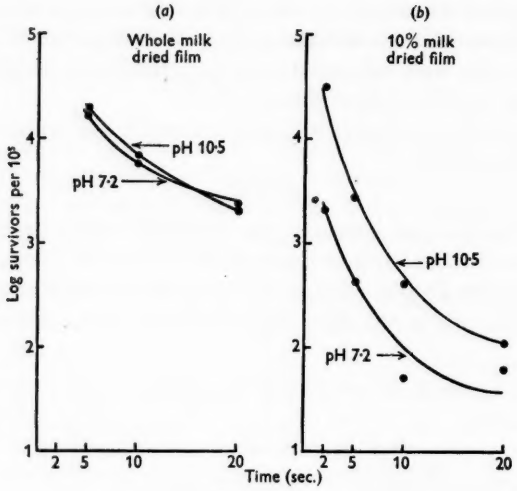


Fig. 2*a*, *b*

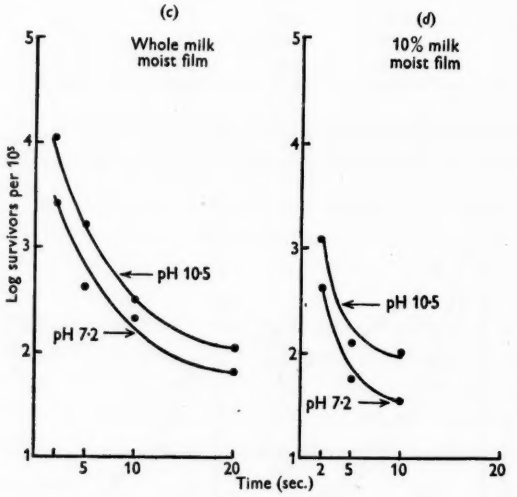


Fig. 2*c*, *d*

This figure shows that there is no advantage in using a solution of low pH on a dried whole-milk film. Either by reducing the milk content or by retaining the film in the moist state not only is the process of disinfection accelerated, but the efficiency of the neutral solution becomes more pronounced.

Table 2 shows the survivors after treatment with neutral hypochlorite expressed as a percentage of those surviving treatment with the alkaline solution. It will be seen from this that except in the dried whole-milk film the neutral solution acts much more rapidly in the first few seconds of the treatment and retains its advantage throughout the test period.

Table 2. *Survivors of treatment with neutral hypochlorite shown as a percentage of survivors of alkaline hypochlorite*

	Time of treatment (sec.)				
Nature of milk	2	5	10	20	40
Dried whole milk	—	83	85	115	105
Dried 10% milk	6·8	16	12	55	—
Moist whole milk	14·0	26	68	59	—
Moist 10% milk	3·3	45	35	—	—

There is no way of comparing the effect of the degree of hydration of the film with the milk content, but in Fig. 2(*b*) and (*c*), where the curves are of about the same value, there is a greater pH effect in the dried 10% milk films, indicating that the milk content is of greater consequence than dehydration in modifying the pH effect.

DISCUSSION

Attention has been directed to the first half minute of treatment because it is felt that disinfection must progress rapidly during this period in order to ensure adequate treatment under practical dairy conditions.

With this as a guide, we find a 99·9% kill in moist 10% milk film in 4 sec. when treated with a neutral hypochlorite and in 8 sec. in a solution at pH 10·5. The milk content of these films is much greater than would be encountered in practice after even the most casual rinsing of equipment. In similar films dried the times are 10 and 20 sec. respectively; that is, the relative rates remain unchanged. It would appear from this that the degree of hydration affects the speed of disinfection without altering the pH effect.

On the other hand, these relative rates seem to be changed in the presence of a higher milk content, so that the greater effect of the neutral solution finally disappears with a dried whole-milk film. We suggest that Neave & Hoy's preparations approach this point, and are therefore inappropriate for comparison with milk films found in practice.

Once again the need for thorough cleaning of equipment immediately after use is emphasized by these results.

SUMMARY

Survivor curves show that, under conditions likely to be found in practice, hypochlorite solutions of low alkalinity disinfect milk films on surfaces more rapidly than those of greater alkalinity.

Dehydration of the milk film results in slower disinfection rates by both neutral and alkaline solutions.

Increasing the milk content in the film reduces the rate of disinfection and reduces the effect of the neutral solution to a greater extent than the alkaline.

REFERENCES

(1) JOHNS, C. K. (1934). *Sci. Agric.* **14**, 585.
(2) CHARLTON, D. B. & LEVINE, M. (1935). *J. Bact.* **30**, 163.
(3) COSTIGAN, S. M. (1937). *J. Bact.* **31**, 1.
(4) SCALES, F. M. & KEMP, M. (1939). *J. Milk. Tech.* **2**, 215.
(5) RUDOLPH, A. S. & LEVINE, M. (1941). *Bull. Iowa St. Coll.* no. 150.
(6) JOHNS, C. K. (1934). *Proc. int. Ass. Milk Dlrs'* **27**, Lab. Sect. p. 49.
(7) ANDREWS, F. W. & ORTON, K. J. P. (1904). *Zbl. Bakt.* I. Orig. **35**, 645, 811.
(8) HOLWERDA, K. (1928). *Meded. Dienst. Volksgezondh. Ned.-Ind.* **17**, 251.
(9) COUSINS, C. M. & WOLF, J. (1946). *Proc. Soc. appl. Bact.* no. 1, 15.
(10) NEAVE, F. K. & HOY, W. A. (1947). *J. Dairy Res.* **15**, 24.
(11) RUEHLE, G. A. & BREWER, C. M. (1931). *Circ. U.S. Dep. Agric.* no. 198.

(*MS. received for publication* 10 *May* 1949)

637.132:614.484

396. CHEMICAL STERILIZATION OF DAIRY EQUIPMENT. THE BACTERICIDAL ACTION OF CHLORINE IN THE PRESENCE OF PROTEIN

By G. A. COX AND H. R. WHITEHEAD
Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 3 Figures)

Hypochlorites and other compounds which have a germicidal action by virtue of their content of 'available' chlorine are finding an increasing use in the dairy industry. In large-scale milk-treatment plant, items of equipment which cannot conveniently be subjected to a heat treatment can be efficiently sterilized by the circulation of a chlorine rinse. Hypochlorites have the disadvantage, however, that they react indiscriminately with all compounds possessing free amino groups, and thus protein deposits present in poorly cleaned equipment may neutralize much of the germicidal power of a chlorine compound. Hence the oft-repeated warning to plant operators that equipment which is to be sterilized by a chlorine rinse must first be thoroughly cleaned. On the other hand, it has been pointed out that some of the chloramino compounds formed by the interaction of hypochlorite with protein and protein derivatives may themselves have a definite if slow germicidal action, and it has even been suggested (1) that chlorine-sterilized milk cans containing traces of milk residues may compare favourably after a storage period with similar cans which have been steam sterilized. Wolf & Cousins (2) explain some of the phenomena associated with the destruction of bacteria by hypochlorite in a milk film on a metal surface by postulating that the hypochlorite reacts with the milk to form chloramino compounds which then exert a sterilizing action in a manner somewhat different from that of hypochlorite itself; in particular, the optimum 'kill' takes place under alkaline instead of under acid conditions. There is, however, very little direct evidence on the germicidal action of compounds formed by the interaction of hypochlorite and protein residues in dairy equipment, and it seemed to us that possibly some useful information might be gained by extending some work reported by Wright (3).

REACTIONS BETWEEN HYPOCHLORITE AND AMINO-ACIDS AND PROTEINS

Wright investigated, from the chemical standpoint, the reactions occurring between a fixed amount of hypochlorite and varying amounts of amino-acid or protein. He found that, depending on the relative proportions of the reacting substances, the available chlorine might be partly or wholly reduced or partly or wholly converted into chloramino compounds by combination with the amino groupings of the amino-acids or proteins. The phenomenon is now well known in the chlorination of drinking water, and dosage with chlorine is so adjusted as to obviate the formation of chloramino compounds which have an objectionable flavour. Wright further showed that the reactions involved proceed rather slowly, so that the result observed depends upon the time allowed for reaction. Some of the chloramino compounds are unstable, particularly when the proportion of

protein to hypochlorite is large. The most clear-cut results were obtained with glycine. Wright's graph showing the results obtained after a 5 hr. reaction period between hypochlorite and glycine is reproduced in Fig. 1.

With proportions corresponding to point *B* on the graph, all the hypochlorite is reduced by the glycine. At points along *AB* part of the hypochlorite is reduced and part remains in the free state. At points along *BC* part of the hypochlorite is reduced and part is

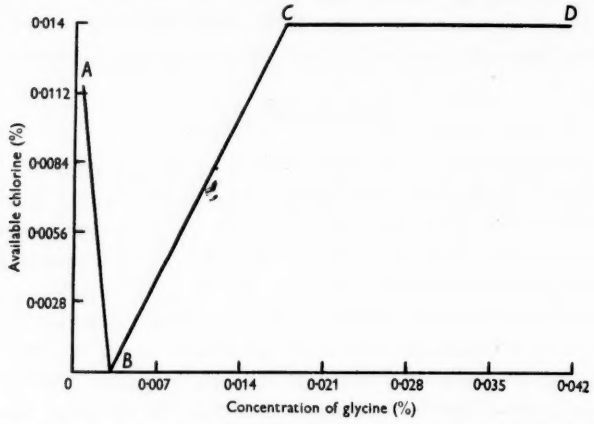


Fig. 1. Effect of glycine in reducing the available chlorine of a solution of hypochlorite (5 hr. contact). (After Wright.)

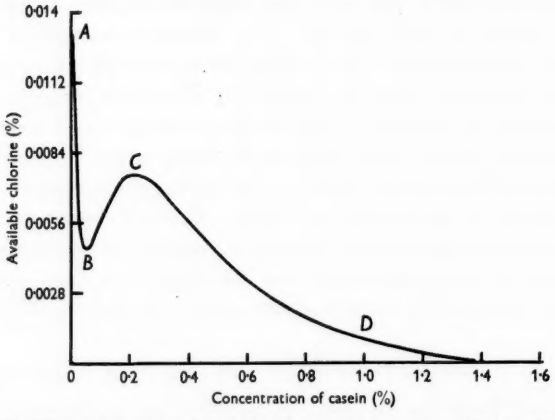


Fig. 2. Effect of neutral sodium caseinate in reducing the available chlorine of a solution of hypochlorite (5 hr. contact). (After Wright.)

converted into dichloroglycine (which will react with iodine and thus is estimated as available chlorine). Along *CD* all the hypochlorite is converted into the chloramine compound which shows the same available chlorine as the original hypochlorite. With other amino-acids and proteins the results are not so clear-cut. Fig. 2 gives Wright's results with casein.

Although the first part of the casein curve shows a general similarity to that for glycine, it is to be noted that there is no proportion of hypochlorite to casein at which complete

reduction of the hypochlorite occurs nor any proportion at which all the chlorine is converted to chloramino compound. The drop along *CD* indicates decomposition of the chloramino compound where larger amounts of casein are present.

Assuming that Wright's interpretation of the graphs is correct, it seemed to us that an investigation of the germicidal power of mixtures of hypochlorite and glycine or hypochlorite and skim milk (in place of casein), corresponding approximately to points *A*, *B*, *C* and *D* on the graphs, might give some information on the relative disinfecting power of hypochlorite and chloramino compounds such as might be formed during chlorine treatment of poorly cleaned dairy equipment.

METHODS

Preliminary trials confirmed Wright's findings and enabled a decision to be reached as to suitable concentrations of glycine or skim milk and hypochlorite. The concentration of hypochlorite in various experiments was always within the range 130–150 p.p.m. Glycine concentrations were set at nil (point *A*), 0·0036% (point *B*), 0·016% (point *C*) and 0·042% (point *D*). Skim-milk concentrations were set at nil, 0·4, 2 and 8%. An experiment with hypochlorite and skim milk carried out according to Wright gave a curve as shown in Fig. 3. The shape of the curve is very similar to that obtained with casein and hypo-

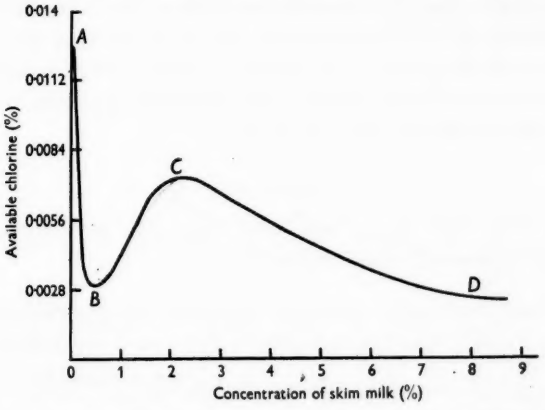


Fig. 3. Effect of skim milk in reducing the available chlorine of a solution of hypochlorite (5 hr. contact, pH 7).

chlorite. The question to be answered was: Do the chloramino compounds presumably present in mixtures corresponding to points *C* and *D* have any disinfecting power, and if so, does such power correspond with the power of hypochlorite at similar available chlorine concentrations?

In the various experiments the required concentrations of glycine or of skim milk (as indicated above) were made up in 5% phosphate buffer and adjusted to the required pH value by additions of alkali or acid. Allowance was made for the change in reaction which would be caused by the subsequent addition of hypochlorite. After the solutions had been sterilized in the autoclave, a final check of the pH value was made, and then an amount of clear hypochlorite solution (prepared from bleaching powder) sufficient to give a final concentration of 130–150 p.p.m. in the mixture was added. The mixtures were allowed to

stand for 5 hr. at room temperature to enable the reaction to proceed to the stage indicated by the graphs. The mixtures were then distributed in sterile reaction tubes, and a modified Rideal-Walker test was carried out to determine disinfectant action against a pure strain of *Str. cremoris*. The test organism was grown on agar plates for 1 day at 30° C. and washed off with sterile buffer solution under conditions standardized as far as possible. Subcultures from the reaction tubes after contact between the bacteria and the test mixtures for 2½ min., 10 min., 1 hr. and 24 hr. (at room temperature, approximately 60–65° F.) were made into tubes of sterilized skim milk, which were then incubated at 30° C. Survival or non-survival of the bacteria was determined either by observation of clotting of the milk or (if clotting was delayed) by examination of smears under the microscope. In order to obtain a more detailed picture of disinfecting power, the mixtures in each experiment were tested, as described above, both in the undiluted form and also after dilution to 1/10, 1/100 and 1/1000 strength in buffer solution. The concentration of available chlorine in the mixture at the time of the start of the tests for disinfecting power was determined by iodometric titration with thiosulphate.

The experiments were first carried out at a pH of about 7·0 and were then repeated at a pH of about 9·5 in order to determine the effect of alkaline conditions on the formation of chloramino compounds and upon their disinfectant action. Charlton & Levine (4) showed that monochloroamine is equal to hypochlorite in germicidal value at pH 9·5 but less germicidal at lower pH values. An attempt was made to repeat the experiments at pH 5, but the results indicated that the hypochlorite was too unstable at this reaction, and that it did not combine with the glycine in the manner indicated in Fig. 1. Control experiments showed that *Str. cremoris* was not significantly reduced in numbers by contact with the 5% phosphate buffer at pH 7·0 or 9·5 for 24 hr.

RESULTS

The experiments both with glycine and with skim milk were repeated many times. Tables 1–4 give the results of typical experiments.

Table 1. *Disinfectant action of mixtures of glycine and hypochlorite at* pH 7·0 (*5 hr. preliminary contact between glycine and hypochlorite*)

Contact time in disinfection test	Dilution of reaction mixture	Point on graph (Fig. 1) *A*	*B*	*C*	*D*
2½ min.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
10 min.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
1 hr.	N	−	+	−	−
	1/10	−	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
24 hr.	N	−	+	−	−
	1/10	−	+	−	−
	1/100	+	+	+	+
	1/1000	+	+	+	+
Available Cl in reaction mixture (p.p.m.)		140	7	106	130

+ =growth of test organism. − =no growth, i.e. disinfectant action.

Table 2. *Disinfectant action of mixtures of glycine and hypochlorite at* pH 9·5 (5 *hr. preliminary contact between glycine and hypochlorite*)

Contact time in disinfection test	Dilution of reaction mixture	Point on graph (Fig. 1) *A*	*B*	*C*	*D*
2½ min.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
10 min.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
1 hr.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
24 hr.	N	−	+	−	−
	1/10	−	+	+	+
	1/100	−	+	+	+
	1/1000	+	+	+	+
Available Cl in reaction mixture (p.p.m.)		130	33	130	136

Table 3. *Disinfectant action of skim milk and hypochlorite mixtures at* pH 7·0 (5 *hr. preliminary contact between skim milk and hypochlorite*)

Contact time in disinfection test	Dilution of reaction mixture	Point on graph (Fig. 3) *A*	*B*	*C*	*D*
2½ min.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
10 min.	N	−	−	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
1 hr.	N	−	−	−	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
24 hr.	N	−	−	−	+
	1/10	−	−	−	+
	1/100	−	+	+	+
	1/1000	+	+	+	+
Available Cl in reaction mixture (p.p.m.)		132	30	70	24

Table 4. *Disinfectant action of skim milk and hypochlorite mixtures at* pH 9·5 (5 *hr. preliminary contact between skim milk and hypochlorite*)

Contact time in disinfection test	Dilution of reaction mixture	Point on graph (Fig. 3) *A*	*B*	*C*	*D*
2½ min.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
10 min.	N	−	+	+	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
1 hr.	N	−	+	−	+
	1/10	+	+	+	+
	1/100	+	+	+	+
	1/1000	+	+	+	+
24 hr.	N	−	−	−	−
	1/10	−	+	−	+
	1/100	−	+	+	+
	1/1000	+	+	+	+
Available Cl in reaction mixture (p.p.m.)		142	47	63	47

From a consideration of the results given in the tables, the following salient points emerge:

(1) Where mixtures of glycine and hypochlorite are allowed to react for 5 hr. the disinfecting power of the resulting solutions bears some relationship to the available chlorine present as estimated by iodometric titration. In the mixtures containing larger proportions of glycine, where the available chlorine is presumably present as dichloroglycine, disinfecting action is slower both at pH 7·0 and 9·5, and takes effect only after a longer time of contact with the bacteria, viz. 1 hr. (pH 7·0) or 24 hr. (pH 9·5) as compared with 2½ min. for hypochlorite.

(2) There is no evidence under the conditions of these experiments that the germicidal power of dichloroglycine is higher at pH 9·5 than at pH 7·0. The indications are rather in the opposite direction.

(3) With mixtures of skim milk and hypochlorite, the results both from a chemical and from a bacteriological standpoint are less clear-cut, as would be expected on the basis of Wright's results. Nevertheless, there is still an indication that the chloramino compounds presumably formed by interaction of the hypochlorite and protein do exert some germicidal action. As with the glycine mixtures this action is evidently slower than that exerted by free hypochlorite. With the skim-milk mixtures there were often in the different experiments slight discrepancies between the disinfecting power of solutions and their available chlorine content. For instance, in Table 3 the mixture corresponding to point *B* and containing 30 p.p.m. of available chlorine has a slightly more powerful germicidal action than the mixture corresponding to point *C* containing 70 p.p.m. available chlorine. Is it possible that some of the available chlorine at point *B* is still present as free hypochlorite?

(4) There is no evidence in the experiments with skim milk of an enhanced germicidal action at pH 9·5 as compared with pH 7·0. But the plan of the experiments was not such that small differences in germicidal power would necessarily be detected. This is evidenced by the fact that hypochlorite itself in the absence of skim milk shows no lowering of efficiency at pH 9·5 as compared with pH 7·0. The estimations of available chlorine in the reaction mixtures afford slight evidence of a greater stability of the chloramino compounds at pH 9·5 where a higher proportion of skim milk is present.

DISCUSSION

To relate the results of experiments, such as those described above, to disinfection by hypochlorite in dairy practice is obviously very difficult. The experimental conditions were highly artificial, and one has to be very cautious in drawing conclusions in relation to actual practice. There is undoubtedly some evidence that compounds formed by interaction of hypochlorite with proteins and protein derivatives have a germicidal power bearing some relationship to their content of available chlorine, and there is evidence that they act more slowly than free hypochlorite. It is clear also that where protein is present in excess the chloramino (or chloroprotein) compounds either are not formed or are unstable, and thus the germicidal value of the mixture is low. It may be permissible therefore to draw a tentative conclusion in relation to practical disinfection in the dairy or dairy factory. Since the germicidal value of the chloroproteins formed during the chlorine sterilization of imperfectly cleaned equipment would depend on the relative proportions of hypochlorite and protein present, the effect in practice would be very

uncertain and variable. The amount of protein residue left on an imperfectly cleaned surface obviously could not be controlled and would in many cases be such as to result in a high proportion of protein to hypochlorite and hence a low disinfecting power. It is possible, however, that the result in a protein film on a metal surface would not be the same as the result in solution, and here again caution is necessary in interpreting the results.

SUMMARY

1. Hypochlorite was allowed to react with glycine and with skim milk in various proportions, and the germicidal power of the resultant mixtures was determined.

2. The results indicated that chloramino compounds formed by interaction of hypochlorite and protein or amino-acid have a definite but slow disinfecting action against *Str. cremoris*.

3. The chloramino compounds did not appear to be more strongly germicidal at pH 9·5 than at pH 7·0 under the conditions of the experiments.

REFERENCES

(1) Scarlett, C. A. (1947). *Dairy Industr.* **12**, 430 (discussion on paper).
(2) Wolf, J. & Cousins, C. M. (1946). *Nature, Lond.*, **158**, 755.
(3) Wright, N. C. (1926). *Biochem. J.* **20**, 524.
(4) Charlton, D. B. & Levine, M. (1937). *Bull. Iowa Engng Exp. Sta.* no. 132.

(*MS. received for publication* 31 *May* 1949)

637.127.6

397. A BURETTE FOR RAPID CHECKING OF GERBER BUTYROMETERS

By R. M. DOLBY

Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 1 Figure)

The calibrations of Gerber butyrometers are usually checked by running mercury from a burette into the butyrometer and comparing the readings on the burette with those on the butyrometer. Burettes normally supplied for this purpose have a jet which is too short to reach more than a short distance into the butyrometer. As the mercury has to fall several inches after leaving the jet, globules of mercury are scattered round the upper part of the butyrometer and air bubbles are included in the mercury in the lower part. Two sources of error are thus introduced into the calibration.

These difficulties can be overcome if the jet of the burette is drawn out to form a long capillary which will pass right down the graduated tube of the butyrometer into the bulb at the bottom. The length of this jet brings the burette to an inconvenient height for reading, but this disadvantage can be overcome by connecting the jet to the burette by an S-shaped tube as shown in Fig. 1. The tip of the jet can now be only 1 or 2 in. below the stopcock of the burette. The S-shaped tube may be joined to the burette by a piece of thick-walled rubber tubing. If the joint is level with the tip of the jet it will not be subjected to either a positive or a negative head of mercury and so will not cause error in the volume delivered. The jet is formed by drawing out a length of tubing of about 8 mm. outside diameter and 3 mm. bore to form a capillary 2–2·5 mm. outside diameter and 10 cm. long.

The burette may be one supplied for the purpose and graduated to correspond to percentage fat on the butyrometer, or may be an ordinary 1 or 2 ml. burette, provided that the length occupied by 1 ml. on the burette is at least 15 cm. (the volume occupied by 1% fat on a milk butyrometer is 0·125 ml.). The burette is connected via its double-bored stopcock to a mercury reservoir *R*, which is also connected through a second stopcock *B* to the S-shaped tube below the burette.

The butyrometer to be calibrated is carried on a support which slides on two vertical rods and can be clamped in any position. A burette clip soldered to the sliding support and closing on one of the vertical rods provides a convenient clamp. A reading lens *L* is supported at such a height that it is focused on the scale of the butyrometer 1 or 2 mm. below the tip of the jet.

Before commencing operations it is desirable to calibrate the burette itself by weighing the mercury delivered. The burette corrections are entered on a card mounted behind the burette.

The checking of a butyrometer is carried out as follows: The butyrometer is clipped on to a sliding support and is raised until the jet of the burette reaches into the bulb of the butyrometer. Mercury is run into the bulb by opening stopcock *B*. While the bulb is filling, the burette is filled and adjusted to zero by stopcock *A*. Stopcock *B* is closed when

the mercury in the butyrometer reaches the level of the lowest graduation as seen through the reading lens *L*. Mercury is now run in from the burette and the butyrometer is lowered as the mercury rises until the next butyrometer graduation which it is desired to check is reached. The burette reading is noted and any correction is applied. The process is repeated until the butyrometer has been checked at the desired number of points.

With this apparatus, butyrometers can be checked very expeditiously, and any which fail to reach the required standard of accuracy are readily detected.

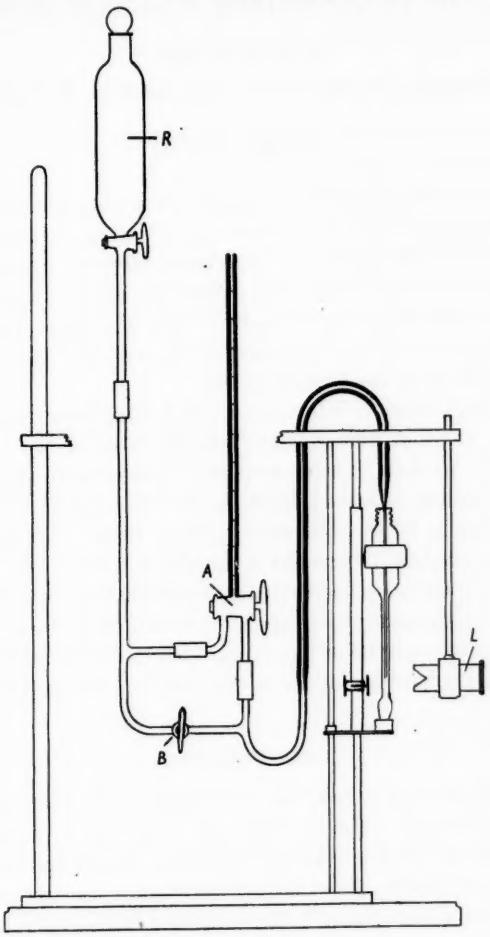


Fig. 1

SUMMARY

The burette is provided with a long capillary jet which can pass down the graduated tube of the butyrometer into the bulb. Splashing of mercury and inclusion of air bubbles are thus avoided. To reduce the overall height, the jet is connected to the burette by an S-shaped tube. The butyrometer is carried on a support which can slide vertically.

(*MS. received for publication* 4 *April* 1949)

637.221

398. THE PROPERTIES OF NEW ZEALAND BUTTERS AND BUTTERFATS

II. THE RELATION OF HARDNESS OF NEW ZEALAND COMMERCIAL BUTTER TO COMPOSITION OF THE BUTTERFAT

By R. M. DOLBY

Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 8 Figures)

Considerable variations in hardness are commonly found when a line of butters is examined, even where all the samples have been drawn from the one factory. While the greatest differences occur between samples taken at different parts of the season, the day to day fluctuations are quite appreciable and may exceed the changes that can be produced by normal variations in manufacturing methods (1). It is of interest, therefore, to determine to what extent changes in the composition of the raw material will account for the variations in the hardness of the finished butter.

It has been shown by Coulter & Hill (2) in the U.S.A. that there is a significant correlation between the iodine value of butterfat and the hardness of the butterfat and of the corresponding butter. Workers in other countries have come to similar conclusions. The wide differences in hardness between Danish and New Zealand butters could not, however, be satisfactorily explained by the difference in iodine value of the butterfats (Lyons (3)).

A survey of the composition of butterfat in samples of butter drawn at monthly intervals over 4 years from nine factories distributed throughout New Zealand has been made by Cox & McDowall (4). The present paper details the results of hardness determinations made during three years on the samples of butter analysed by Cox & McDowall, and shows the relationship between the hardness of the butter and the composition of butterfat.

EXPERIMENTAL

The source of the samples has already been described (4). The samples were forwarded by parcel post from the factories to the Institute, and on receipt were placed in a refrigerator at 5° C. After 2–3 days a 1 lb. block was cut from each sample and was held in a constant-temperature cabinet at 12·5° C. for at least 2 days before the measurements of hardness were made. Hardness was determined by the sectility method (6). The results were expressed as the load in grams required to produce a rate of cutting of 0·001 cm./sec. (F 0·001). These results represent the 'practical yield value' of the butter. The slope n (which has been considered to represent 'structural viscosity') (7) was also recorded. The value of n showed no systematic changes with either season or factory, and it was not correlated with any of the other variables except the hardness of the butter. The mean value of n for the 324 samples investigated was 11·0 and the standard deviation $\pm$ 1·6. As the variations in n appeared to be random and may have been largely due to experimental error, they have not been considered further in this paper.

PRELIMINARY INVESTIGATION OF THE RELATION BETWEEN HARDNESS AND COMPOSITION

In order to determine which of the four variables (iodine value, Reichert value, saponification value and softening point) should be included in the complete analysis of results, a preliminary graphical investigation was made of 1 year's results (1939-40) season.

When the hardness values were plotted against the corresponding iodine values, a definite correlation was exhibited, the hardness decreasing with increasing iodine value. The relationship was curvilinear (cf. Coulter & Hill (2)). If, however, the reciprocal of hardness was plotted against iodine value, the average curve was rectilinear, as shown in Fig. 1, in which iodine values are taken as abscissae, and values of $\frac{1}{F_{0\cdot001}} \times 1000$ (referred to as 'softness') as ordinates. Group averages for hardness are shown as crosses connected by

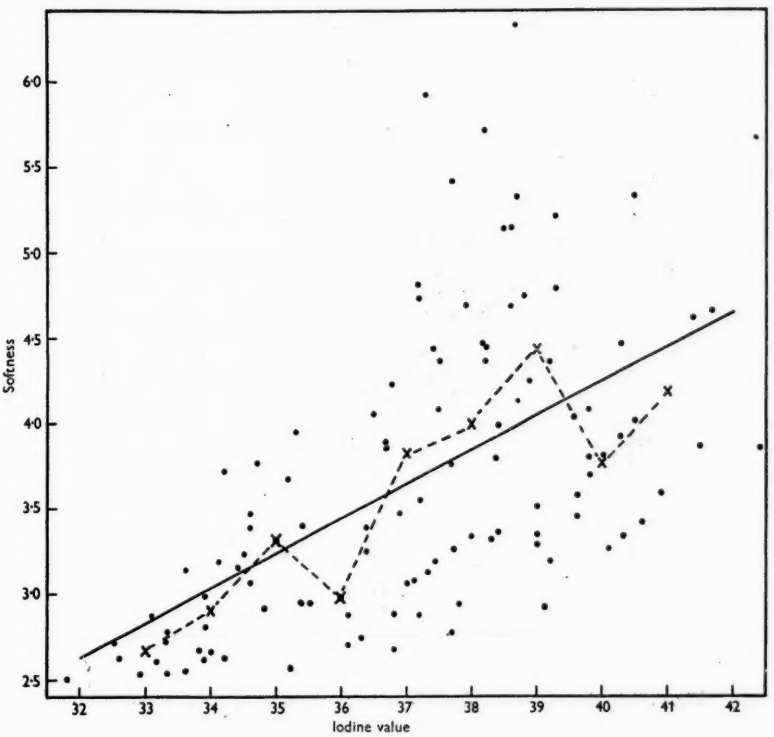


Fig. 1. Relation between softness of butter and iodine value of butterfat.

a broken line. The general trend is indicated by the straight line which was fitted by the method of least squares. It is evident that there is a definite correlation between the softness of the butter and the iodine value of the butterfat.

In Fig. 2, values for softness have been plotted against softening point in the same manner as was done for iodine value. Here again a definite correlation is seen to exist. A similar though less definite correlation was found between softness and saponification value, but there appeared to be little or no correlation between softness and the Reichert value. It was found that there was a high correlation between iodine value and saponifica-

tion value, while there was a less obvious correlation between iodine value and Reichert value. It is therefore possible that the correlation found between saponification value and softness is due to the fact that each is correlated with iodine value. The correlation independent of iodine value may be illustrated by plotting the deviations from the straight line in Fig. 1 against one of the other variables.

In Fig. 3 the deviations have been plotted against softening point. It is evident that there is a correlation between softening point of butterfat and softness of butter independent of the iodine value of the butterfat. In other words, variations in softening point explain some of the variations in softness not explained by iodine value. In Figs. 4 and 5,

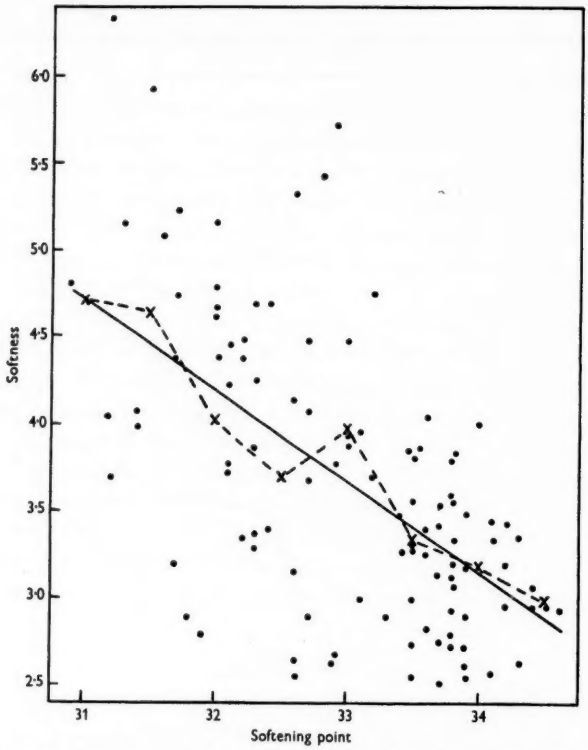


Fig. 2. Relation between softness of butter and softening point of butterfat.

the same method has been applied for saponification value and for Reichert value respectively. It would appear that in neither case is there a significant correlation with softness independent of iodine value.

From the above considerations it was concluded that the two variables, iodine value and softening point, would give useful information on the effect of composition of butterfat on softness of butter, and that little additional information would be gained by including the data for saponification value and Reichert value. Only three variables, viz. iodine value, softening point and softness, were retained, therefore, in making a statistical analysis of the data.

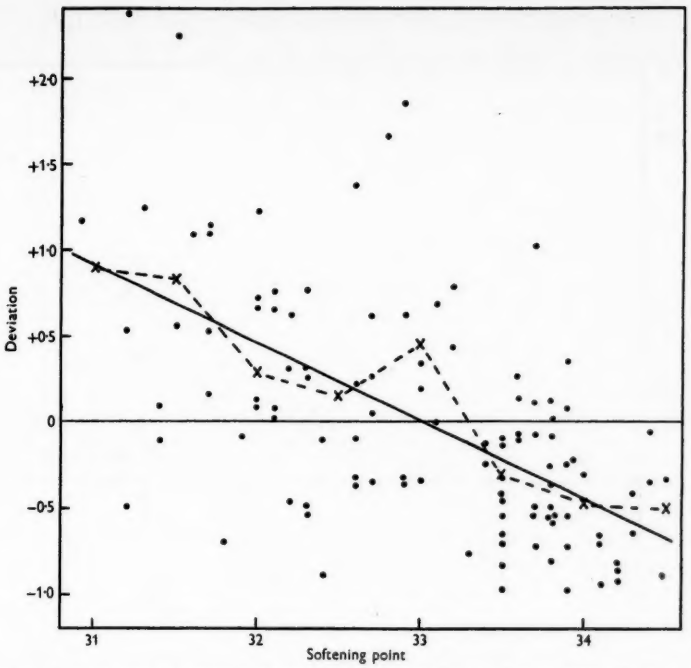


Fig. 3. Deviation of softness of butter from straight line in Fig. 1 plotted against softening point of butterfat.

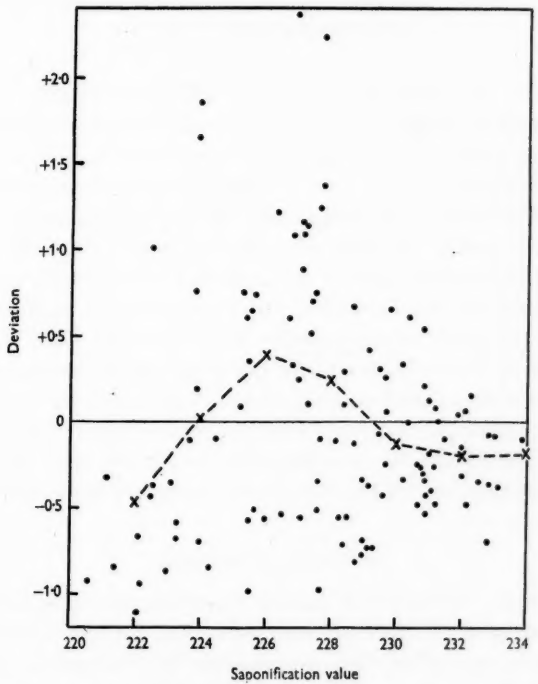


Fig. 4. Deviation of softness of butter from straight line in Fig. 1 plotted against saponification value of butterfat.

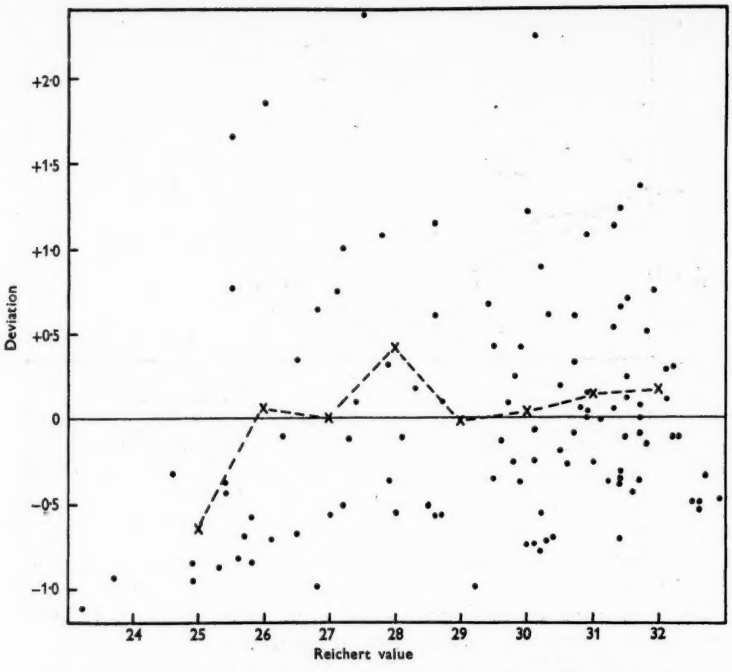


Fig. 5. Deviations of softness of butter from straight line in Fig. 1 plotted against Reichert value of butterfat.

SEASONAL TRENDS IN 'SOFTNESS' OF BUTTER

In Fig. 6, the whole of the results for the 3 years for the softness of butter (reciprocal of the hardness) have been plotted as a scatter diagram against the months in which the samples were taken. The averages for each of the months have been connected by a broken line. The values for softness of the individual samples show a wide scattering on each side of the mean value for each month. It will be seen that in the spring months, July, August and September, the softness of the butter is much above the average for the year. The butter becomes rapidly harder in October and continues to increase in hardness until December. From January to June the butter gradually becomes softer. The average curve closely follows that given by Cox & McDowall for the iodine values for the butterfat, the only noticeable difference being that iodine values do not fall markedly until November, while the softness of the butter falls steeply in October. Examination of the curve for softening points given by these authors fails to explain this discrepancy, since the average softening points are at a minimum in October and reach a maximum in January.

STATISTICAL ANALYSIS

A statistical analysis by the method of multiple regression was made for the three variables, softness (Y), iodine value (X_1) and softening point (X_2), the data being grouped according to factories, months and years. The computations for the analysis are summarized in Table 1. The method for calculating interactions follows that given by Snedecor ((7), p. 313).

In Table 2 are shown the means and standard deviations of the three variables for the 324 samples of butter.

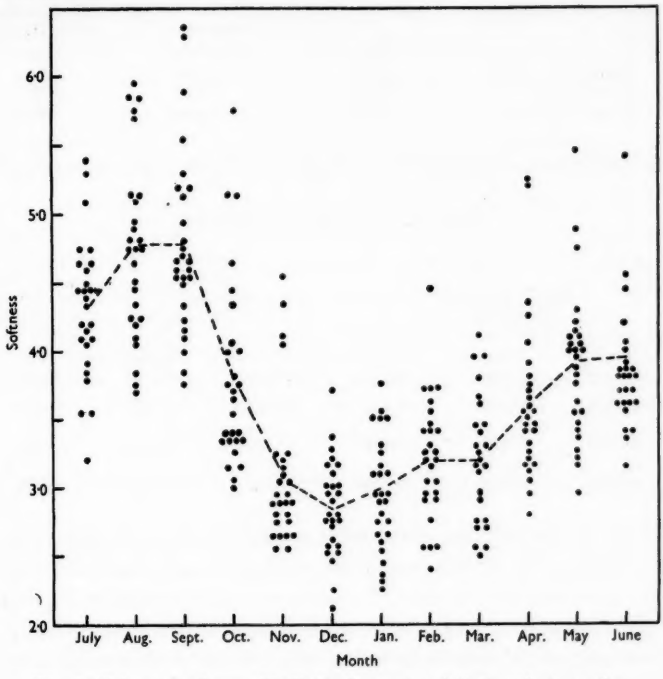


Fig. 6. Values of softness of individual samples plotted against months.

Table 1. *Analysis of covariance and regression*

Source of variation			Sums of squares and products		
			Iodine value	Softening point	Softness
Factory × Month	D.F.	Variate	X_1	X_2	Y
Factory	8	X_1	320·45	17·08	−24·72
Month	11		1510·50	−50·20	355·03
Year	2		4·27	−1·42	0·12
Factory × Month	88		153·70	−8·74	37·14
Factory × Year	16		8·97	−0·66	6·15
Month × Year	22		48·07	−6·73	12·98
Remainder	126		63·99	−7·29	13·69
Total	273		2109·95	−57·96	400·39
Factory	8	X_2	—	41·41	−31·40
Month	11		—	125·31	−70·69
Year	2		—	0·91	−0·36
Factory × Month	88		—	9·52	−3·55
Factory × Year	16		—	1·40	−0·61
Month × Year	22		—	17·07	−2·96
Remainder	126		—	16·47	−2·73
Total	273		—	212·09	−112·30
Factory	8	Y	—	—	31·71
Month	11		—	—	133·94
Year	2		—	—	0·24
Factory × Month	88		—	—	26·35
Factory × Year	16		—	—	5·20
Year × Month	22		—	—	7·49
Remainder	126		—	—	21·52
Total	273		—	—	226·45

Table 2. *Means and standard deviations*

	Mean	Standard deviation	Coefficient of variation (%)
Softness	3·700	±0·837	22·62
Iodine value	37·180	±2·557	6·88
Softening point	32·951	±0·810	2·46

Correlation coefficients calculated from the data in Table 1 are set out in Table 3.

Table 3. *Correlation coefficients*

Source of variation	Total correlation coefficients			Standard partial regression coefficients		Multiple correlation coefficients	
	r_{12}	r_{y1}	r_{y2}	$b'_{y1\cdot2}$	$b'_{y2\cdot1}$	R^2	R
Total	−0·0867	0·5791	−0·5123	0·5389	−0·4656	0·5502	0·7418
Within factories	−0·1358	0·7199	−0·4438	0·6720	−0·3525	0·6402	0·8002
Within months	−0·0340	0·1911	−0·4643	0·1756	−0·4585	0·2465	0·4965
Remainder	0·2246	0·3689	−0·1450	0·3452	−0·0654	0·1402	0·3744

The coefficients in the first line of the table are derived from the total sums of squares and products and show the correlations which exist among the variables when no account is taken of the grouping into months, factories, etc. The correlation r_{12} between iodine value (X_1) and softening point (X_2) is too small to be significant. That is to say, the softening points of the samples were quite independent of their iodine values. This conclusion is of interest, as it might be expected that butterfat with a high iodine value would have a low softening point. Further comments on this observation will be made later.

The correlations between softness and iodine value (r_{y1}) and between softness and softening point (r_{y2}) are both highly significant. A comparison of the values for the standard partial regression coefficients shows that change in iodine value had a rather greater effect on the softness of the butter than did change in softening point. The value for R^2 (0·55) shows that iodine value and softening point together accounted for 55% of the variation in softness of the butter samples.

The second line of Table 3, obtained by deducting sums for factories from total sums, indicates the relationship which would occur in samples of butter from a single factory. Since differences between factories have been eliminated, iodine value and softening point account for a greater proportion of the variance in softness ($R^2 = 0{\cdot}64$) than where the data are taken as a whole. In the case of single factories, iodine value accounts for a considerably greater proportion of the variation in softness than does softening point. It would appear that differences in softening point result from district differences as well as from seasonal variations, while changes in iodine value are mainly seasonal variations. The correlation between iodine value and softening point within factories is greater than in other cases, but although it surpasses the 5% value of significance it does not reach the 1% level.

The figures in the third line of Table 3 are derived by subtracting sums for months from total sums of squares and products. The correlations arrived at represent the average of those for each month and indicate the relationship to be expected in any one month.

As changes in iodine value and softening point in any one month are small, a greater proportion of the variance must be attributed to other factors. The value for R^2 (0·24) is thus much lower than that for the total variation. The values for b' show that in any one month differences between factories in softness of butter were due much more to differences in softening point of the butterfat than to differences in iodine value. This confirms the observation made in the preceding paragraph.

In the fourth line of Table 3 are shown the correlations calculated from remainder sums. In these sums all differences due to months, factories and years and their interactions have been eliminated, and the correlations show what relationships would be expected if all the samples had been made in one factory in one month. As changes in iodine value and softening point have been largely eliminated, these variables explain only a small portion (14%) of the variation in softness. The small variations in softness occurring under these conditions ($Sy^2 = 21{\cdot}52$ out of a total for $Sy^2 = 226{\cdot}45$—Table 1) therefore measure the random variations in softness to be expected in a factory over a period of a few days.

From the total figures we can derive the total regression equation

$$\hat{Y} = 13{\cdot}00 + 0{\cdot}1774\, X_1 - 0{\cdot}4826\, X_2,$$

which shows the relation between softness, iodine value and softening point for the 324 samples.

Further information on factors affecting the softness of butter may be gained by calculating the estimated values of softness by means of the total regression equation and comparing them with the values actually found.

An analysis of variance of these errors of estimate ($Y - \hat{Y}$) is given in Table 4. The method followed in calculating percentages of total variance is that given by Snedecor ((7), p. 307).

Table 4. *Analysis of variance of errors of estimate*

Source of variation	D.F.	Sum of squares	Mean square	Percentage of total variance
Factories	8	26·96	3·37**	59·17
Months	11	25·13	2·28**	36·34
Years	2	0·44	0·22 N.S.	0
Factories × Months	88	18·29	0·21**	1·70
Factories × Years	16	3·15	0·20 N.S.	0·10
Months × Years	22	6·65	0·30**	0·40
Remainder	176	21·10	0·12	2·30
	323	101·72		100

** Significant at 1% level. N.S. Not significant.

This table indicates that, of the variance in softness which is not explained by variations in iodine value and softening point, 59% is due to differences between one factory and another and 36% to differences between one month and another, these differences being distinct from any effects of iodine value and softening point. The three interactions Factories × Months, Factories × Years and Months × Years account together for a further part of the variance. The term Factories × Months, which accounts for 1·7% of the variance, indicates that the seasonal variations are not quite the same at different factories, e.g. seasonal changes in the South Island districts tend to occur a few weeks later than in the North Island. The term Months × Years indicates differences in seasonal changes between one year and another, e.g. maximum softness in one year may occur in

September while in another year it may occur in August. The term Factories × Years is not significant. Evidently differences between factories were consistent from year to year.

The Remainder term, amounting to 2·3% of the variance not explained by X_1 and X_2, includes all random deviations in softness of butter and all experimental errors in the determination of softness, iodine value and softening point.

The variations due to months and to factories have been investigated further by graphical methods. The errors of estimate from the total regression equation (i.e. the difference between the estimated and actual values for softness) have been calculated for each of the samples and plotted against months in Fig. 6. It will be seen that the softness of spring butter (July–September) has been only partly explained by the high iodine value and low

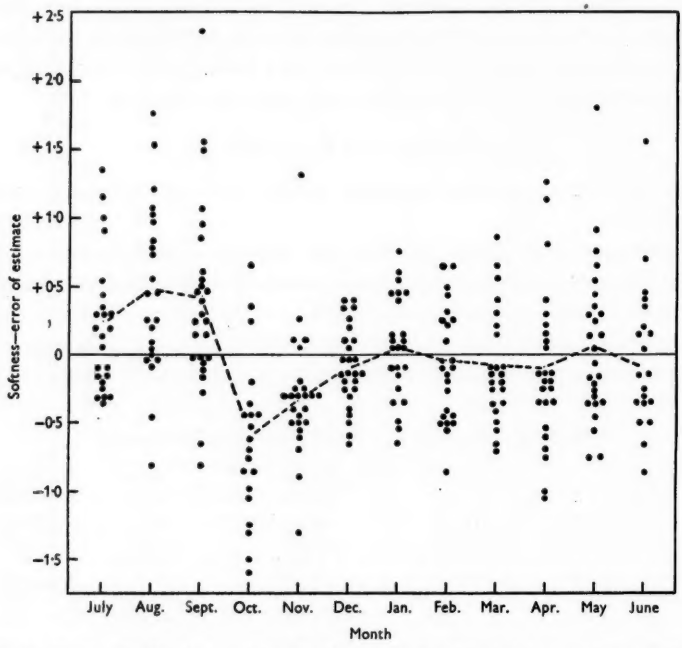


Fig. 7. Errors of estimate in values of softness calculated by total regression equation plotted against months.

softening point of butterfat produced during these months. There is thus some additional factor which tends to cause spring butter to be soft. It is not likely that manufacturing methods contribute to this, as churning temperatures and other conditions in processing the cream are adjusted to offset as far as possible the softness of the spring butter.

During October and November the butter is harder than would be expected from the iodine value and softening point of the butterfat. The low value in October is consistent with the observation made earlier that, while softness of butters falls rapidly during October, iodine value remains high and softening point low.

From December onwards the average error of estimate is small, indicating that the softness of the butter corresponds well on the average with that expected from the iodine values and softening points of the butterfat.

An adjustment can be made to the values of errors of estimate for the mean monthly changes shown in Fig. 6, by subtracting from the error for each sample the average error for the particular month. Such adjusted points have been plotted in Fig. 7 against factories. The adjustment affects only the scatter of points and not the mean for each factory. This graph shows how butter from each factory varied in softness after making allowance for iodine value and softening point and for regular monthly deviations due to other causes.

It will be seen that the butter from some factories is consistently softer than that from others, even when these allowances have been made. These differences may be due either to different manufacturing conditions in the various factories or to district differences in

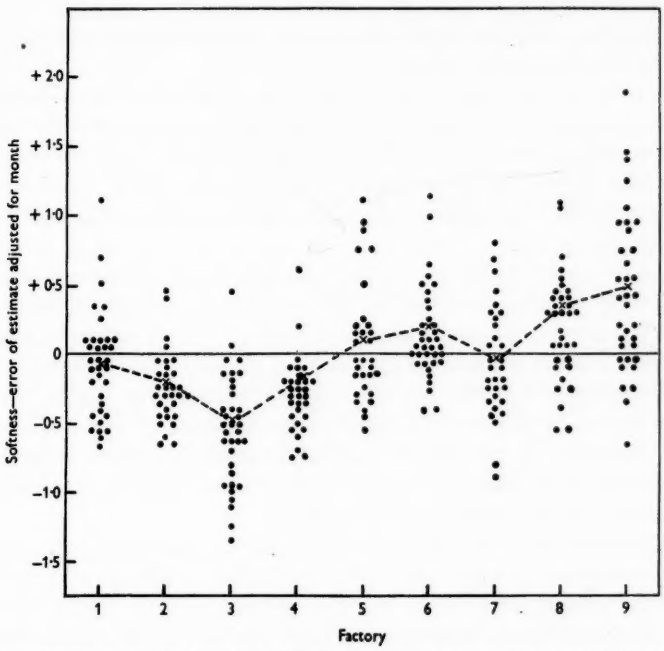


Fig. 8. Errors of estimate in values of softness corrected for average monthly deviations plotted against factories.

the raw material. Factories 8 and 9 in the South Island made butter which was considerably softer than that from the North Island factories. The iodine value and softening point of the butterfat explained only a part of this difference. It is likely that the climatic differences, differences in the feeds available and possibly breed differences were largely responsible for this difference. That there are large district differences between the South and North Islands is evident from Cox & McDowall's results ((4), Fig. 6, p. 385), which show that even the seasonal trends in butterfat composition differ considerably, especially during the winter months. The observation that factories 2, 3 and 4 (North Auckland and Waikato) made harder butter than the other factories after allowing for iodine value and softening point may also be partly due to district differences.

DISCUSSION AND CONCLUSIONS

It has been shown that, in the samples investigated, iodine value and softening point of butterfat were correlated with the softness of the corresponding butter. The iodine value and softening point of butterfat were, however, independent of each other. The observation that an increase in iodine value did not bring about a decrease in softening point of the fat requires some explanation. Irregularities in the experimental determination of softening point cannot have been the cause, since softening point was almost as well correlated with butter softness as was iodine value. Two possible explanations may be suggested:

(1) That increase in iodine value is accompanied by a compensatory change in the proportions of the lower saturated acids so that the average melting-point of the glycerides remains much the same.

(2) That softening point is determined more by glyceride structure than by the proportions of the individual fatty acids.

Some support for the first suggestion is found in the observation that with an increase in iodine value in the samples, there was a definite tendency for a decrease in the saponification value and a less definite tendency for a decrease in the Reichert value, indicating a decrease in the proportion of the lowest members of the fatty acid series. It seems likely that changes in glyceride structure will also affect the softening point, so that both explanations suggested may play a part.

The changes in iodine value and softening point of butterfat have been shown to explain 55% of the variation in softness of the butter samples examined. Of the remaining variation, 95% is attributed either to seasonal variations or to variations between factories. In the former, changes in manufacturing conditions probably play little part, while in the latter, district differences in the raw material are probably at least as important as differences in manufacturing methods between the factories. It can reasonably be concluded from the results that at least 80% of the variations in softness found in the butter samples examined can be attributed to variations in the composition of the butterfat or to physical differences in its dispersion in the cream.

The small part played by manufacturing variations in determining the softness of the butter is indicated further by the extremely small random variations in softness—2·3% of the variation not explained by change in iodine value and softening point. Experimental error in the analytical determinations could easily account for the whole of this residual variation. It is probable, however, that some of the experimental error is included in some of the other components of the variance. For example, the effect of any slight modifications or variations in analytical methods occurring from time to time would be included in either monthly or yearly variations. Even when allowance is made for this possibility, however, it is clear that day-to-day variations in manufacturing conditions must have had a very small effect on the softness of the butters sampled. The day-to-day variations found in hardness of butter must be largely attributed to fluctuations in the properties of the raw matterial.

SUMMARY

Determinations of hardness were made on 324 samples of butter drawn monthly for 3 years from nine factories distributed throughout New Zealand. The results were compared by statistical methods with the iodine values and softening points of the corresponding butterfats which had been determined by Cox & McDowall(4). It was shown that the

softness (reciprocal of hardness) was significantly correlated with both iodine value and softening point, which together explained 55% of the variation in softness observed. Of the remaining variation, 59% was due to seasonal changes other than those in iodine value and softening point and 36% to differences between factories. It is considered that manufacturing variations were responsible for very little of the seasonal variations, and that the differences between factories were due as much to district differences as to manufacturing differences. It is concluded that only a small part of the variation in softness of the butter samples was due to manufacturing variations, and that at least 80% of the variation could be attributed to differences in the raw material.

The thanks of the author are due to Mr G. A. Cox for making his results available and to Mr I. D. Dick, Officer in Charge, Biometrics Section, Department of Scientific and Industrial Research, for advice on the statistical treatment and for computing the data in Tables 1 and 4.

REFERENCES

(1) DOLBY, R. M. (1941). *J. Dairy Res.* **12**, 344.
(2) COULTER, S. T. & HILL, O. J. (1943). *J. Dairy Sci.* **17**, 543.
(3) LYONS, J. (1936). *Econ. Proc. R. Dublin Soc.* **3**, 19.
(4) COX, G. A. & MCDOWALL, F. H. (1948). *J. Dairy Res.* **15**, 377.
(5) DOLBY, R. M. (1941). *J. Dairy Res.* **12**, 329.
(6) DOLBY, R. M. (1941). *J. Dairy Res.* **12**, 337.
(7) SNEDECOR, G. W. (1946). *Statistical Methods*, 4th ed. Ames, Iowa: The Iowa State Collegiate Press.

(*MS. received for publication* 4 *April* 1949)

637.222:612.392.01

399. THE ESTIMATION OF VITAMIN A IN BUTTER

A CRITICAL STUDY OF METHODS

By A. K. R. McDOWELL
Dairy Research Institute (N.Z.), Palmerston North, New Zealand

Extraction of the unsaponifiable matter from the liquid obtained by saponification of a weighed amount of a butter or butterfat sample is commonly accepted as an essential preliminary in the estimation of vitamin A* in butter; and the Carr-Price test, based on the reaction between vitamin A and antimony trichloride, has been found over the last 10 years to be the most satisfactory method of estimation of the vitamin in the unsaponifiable residue (1, 2, 3). The most suitable instrument for the assessment of the transient blue colour of the Carr-Price reaction is a direct-reading photoelectric colorimeter (3). Other workers when estimating vitamin A in butterfat have used the Lovibond Tintometer (4, 5, 6) and the Pulfrich Photometer (7, 8) for this purpose.

Vitamin A may be estimated also by measurement of the intensity of absorption at 325–328 mμ. when the unsaponifiable residue is dissolved in a suitable solvent. The earlier workers in this field, however, realized that some correction must be made for 'irrelevant absorption', i.e. for absorption other than that due to Vitamin A itself. Morton & Heilbron, who, in 1930, were the first to examine the absorption spectra of the unsaponifiable matter of butter, concluded that the ultra-violet absorption was due to (1) carotene, (2) an unidentifiable substance with a maximum absorption at or near 272 mμ, and (3) true vitamin A (9). Later, Gillam, assuming that carotene alone was responsible for irrelevant absorption, corrected the gross reading at 328 mμ by subtracting 1/6·5 times the observed value at 455–460 mμ—the maximum reading for carotene in chloroform (10). Later work has shown that for pure β-carotene the absorption at 325–328 mμ is only 1/20 of that at 450 mμ (11, 12). In the process of extracting the unsaponifiable residue from butterfat, however, some isomerization of β-carotene has usually taken place (13, 14), and these isomers show a much greater absorption in the ultra-violet region than does the pure compound (11, 15). The source of Gillam's carotene is not stated (16), but if it were not isolated from butterfat the isomerization was, fortunately, somewhat similar to that occurring with butterfat carotene. His correction is still in use and, according to a recent paper, 'works well in practice' (17).

Baumann & Steenbock crystallized out impurities from a methyl alcohol solution of the unsaponifiable residue of butterfat at $-72°$ C. (18) (later, the temperature of an ordinary ice-salt mixture was found satisfactory (19)); and they corrected for irrelevant absorption due to carotene by subtracting 10% of the reading at 440 mμ from the gross reading at 328 mμ (20). They also found considerable absorption below 300 mμ, but showed that sterols and fatty acids would not contribute to the absorption at 320–330 mμ (18). They suggested that the interfering agents were oxidation products of carotenoid substances,

* The term vitamin A throughout this paper refers only to true vitamin A and does not include pro-vitamin compounds.

and they discarded all analyses in which the absorption at 280 mμ exceeded that at 325 mμ. In a later paper (21) Gillam has also suggested that oxidative changes during saponification and extraction may be responsible for the compounds exhibiting general absorption in the region 300–350 mμ, and he states that preliminary heating (of milk) rendered the unsaponifiable matter even more susceptible to change. Fraps & Kemmerer crystallized out impurities at −8° C. and corrected for carotene absorption in the ultra-violet (22, 23), but as they used 'purified carotene' their correction may be too small. In their latest method of calculating the vitamin A potency of butterfat these authors make allowance for 'pseudo-vitamin A' by subtracting 0·5 from the total 'spectro-vitamin A', reported in parts per million (24).

Zscheile, Henry, White, Nash, Shrewsbury & Hauge (14) have shown that none of the usual methods, viz. adsorption, use of Lloyd's reagent, acid extraction, or freezing, are effective in removing interfering substances and in yielding a vitamin A extract of which the spectrum corresponds to that of pure vitamin A. They compared results from six butterfats by the spectrophotometric and the antimony trichloride methods of estimation, and calculated that for the unsaponifiable residue dissolved in ether the absorption due to vitamin A itself was only 78% of the gross absorption at 324 mμ.

Neal, Haurand & Luckmann (25) corrected for irrelevant absorption by subjecting butterfat unsaponifiable residues, dissolved in cyclohexane, to ultra-violet irradiation, with complete destruction of vitamin A and carotene. They corrected for the effect of carotene in the original absorption by Gillam's method and assumed that the residual absorption after irradiation (10–25% of the original absorption) was due to non-carotenoid interfering substances. Baumann & Steenbock subjected unsaponifiable residues dissolved in ether to ultra-violet irradiation and to daylight but found very little residual absorption either at 325 mμ or at lower wave-lengths (18).

Morton & Stubbs, using the Beckman Spectrophotometer, have shown with fish-liver oils and vitamin A concentrates that readings of the intensity of absorption at three appropriate wave-lengths—at the maximum for vitamin A or its esters and at wave-lengths on either side of the maximum—will provide sufficient data for calculation of the irrelevant absorption of these compounds (26, 27). The equation is valid only if the assumption of linearity for the irrelevant absorption over the range of the three wave-lengths is justified.

EXPERIMENTAL

Butterfat prepared from butter samples by melting, and then filtering, at 60° C., was used in preference to butter for saponification (13). The saponification process followed closely that developed by Edisbury for margarines (28) and by Goodwin for butters (2), and was as follows: To 8 g. of butterfat were added 10 ml. of redistilled ethanol, 5 ml. of 50% caustic potash solution, and a glass bead. The mixture was boiled gently on a hot plate for 15 min. Water (50 ml.) was added, the mixture was cooled and was extracted once with 50 ml., and then three times with 30 ml. quantities of freshly distilled ether. The combined ether extracts were washed once with 25 ml. of water, once with 25 ml. of 0·5 N-caustic potash and then four times with 25 ml. quantities of water. The ether extract was dried over sodium sulphate, the volume was made up to 100 ml., and appropriate quantities were used for vitamin A or carotene estimation. When vitamin A was to be estimated spectrophotometrically special precautions were taken against contamination from cork and rubber, and from lubricating grease. Flasks and condensers with ground-glass joints were

used for saponification, and the stopcocks of the separating funnels were lubricated with glycerine (22).

VITAMIN A ESTIMATION

(1) *By the Carr-Price test*

Fifty ml. of the ether extract were withdrawn for carotene estimation or for vitamin A estimation by the spectrophotometric method. The remaining 50 ml. were carefully evaporated to dryness first on a water-bath at 50° C., then finally under reduced pressure, and the residue was dissolved in chloroform for the antimony trichloride test. In the absence of a direct-reading photoelectric colorimeter an alternative method of measuring the blue colour was sought. The Pulfrich Photometer gave variations of up to 15% between readings on the same sample, and was therefore not considered satisfactory. The Spekker Absorptiometer with filters OR2 was used at first, as suggested by Innes & Birch (30), with a set drum reading, and the galvanometer reading was recorded. Since the results were somewhat erratic, the usual 'drum-reading' method was adopted. Neutral filters were included on either side of the projector lamp to lower the intensity of the light and to reduce the rate of fading of the blue colour in the antimony trichloride test (31). Here also the results, either with a vitamin A concentrate or with butterfat unsaponifiable residues, were not consistent enough to justify the use of the instrument.

The Lovibond Tintometer with artificial light attachment was found to give the most consistent and reproducible results. The use of the tintometer for the Carr-Price test has been criticized on the grounds that the instrument is 'unsuitable for the measurenent of the particular shade of blue' (32). In practice, however, it has been found that with red and neutral glasses in addition to the blue, workers with normal 'colour sense' will generally agree to within 0·2 blue unit or less, i.e. a maximum variation of 4% at a reading of 5·0–6·0 blue.

Dilution with chloroform to give a reading of 5·0 blue has been suggested (28) as essential for accurate reading of the Carr-Price colour with the tintometer, but it was found more convenient in practice to dissolve the unsaponifiable butterfat residues in the same quantity (2 ml.) of chloroform each time and to pipette out the appropriate quantities of solution—0·2, 0·15 or 0·12 ml.—to give with 2 ml. of antimony trichloride solution a reading between 4·9 and 6·3 blue. Notevarp & Weedon (33) found that though the blue colour varies in intensity according to Beer's law, the blue readings are not strictly proportional to vitamin A concentration. They evolved a formula for fish-liver oil concentrates by which the correct blue reading, e.g. based on a 5·0 blue reading, may be obtained from the actual blue reading. This correction has been found in the present investigation to be valid also for butterfat residues. Readings were confined to the range 5·0–6·2 blue, since matching with the blue glasses is less accurate outside this range. The tintometer glasses must be wiped clean just before use each day, since a film of moisture on the glasses will lead to erroneous results.

Capsules of distilled vitamin A concentrate (P.C.-3, Distillation Products, Inc.) and a highly concentrated vitamin A preparation (British Drug House, labelled 1,000,000 i.u. per g.) were available for standardizing the tintometer. Readings for ethanolic solutions of both compounds on the Beckman Spectrophotometer showed that by Morton & Stubbs's equation neither compound was free from irrelevant absorption. The unsaponifiable residues from both products, however, gave spectrophotometric readings consistent with

those for pure vitamin A alcohol, and chloroform solutions of these residues were used in the standardization test. Addition of known quantities of vitamin A to butterfat unsaponifiable residues—the 'increment method' of Oser, Melnick & Pader (3)—was adopted as the most accurate method of standardization, and consistent results were obtained with a wide variety of butterfats. It was found that

$$\mu\text{g. of vitamin A per g. of butterfat} = \frac{\text{Moore blue units per g. of butterfat}}{5{\cdot}0}.$$

Since the carotene of the butterfat reacts with antimony trichloride, its contribution to the total blue colour in the Carr-Price test must be determined. Previous workers using the tintometer subtracted one-tenth of the Moore yellow units per g. of butterfat from the total blue units per g. (34, 35). If 1 μg. of carotene = 2 yellow units (36), then 1 μg. is equivalent to 0·2 blue unit, or, by the equation above, to 0·04 μg. of vitamin A. As the intensity of the blue colour from β-carotene isomers and antimony trichloride is somewhat higher than that from pure β-carotene alone (37), it was thought advisable in practice to determine the effect of the addition of carotene, isolated from one butterfat, on the blue colour of another. Vitamin A was extracted from petrol ether solutions of a number of residues by shaking at least seven times with 90% methyl alcohol (38). After evaporation of the petrol ether under reduced pressure the carotene residue was dissolved in the chloroform solution of an unextracted residue and the increase in the blue reading was determined. From a number of estimations it was concluded that 1·0 μg. of carotene increased the blue reading by 0·3 blue unit or by 0·06 μg. of vitamin A.

The blue reading expressed as Moore blue units per g. was converted into μg. of vitamin A as follows:

$$\frac{\text{Moore blue units per g.} - 0{\cdot}3 \times \mu\text{g. of carotene per g.}}{5} = \mu\text{g. of vitamin A per g. of butterfat.}$$

This conversion factor gives a lower content of vitamin A than Barnicoat's factor (6), viz.: $\frac{\text{Moore blue units}}{4{\cdot}2}$ or Bartlett's factor (39), viz. $\frac{\text{Moore blue units}}{3{\cdot}9}$; but neither of these workers used pure vitamin A alcohol for standardization of the tintometers. Variations in tintometer conditions, e.g. the use of artificial light as compared with daylight, and the proportions of yellow or neutral glasses used, also modify the results considerably.

(2) *By the spectrophotometric method at* 325 mμ

Intensity of absorption in the ultra-violet region was measured by the Beckman Spectrophotometer with corex cells and tungsten filament lamp. Petrol ether (boiling-point range 55–65° C.) was used in preference to absolute alcohol as a solvent. The spectra of pure vitamin A alcohol in both solvents were found to be almost identical.

Butterfat residues, after evaporation of ether, were dissolved in a sufficient quantity of petrol ether to give a '20%' solution based on the original weight of butterfat taken, and readings were recorded on the spectrophotometer at 325 mμ and also at 310·5 and 335·5 mμ, i.e. at the maximum reading and at six-sevenths of the maximum on either side for pure vitamin A alcohol in petrol ether solution. If Morton & Stubbs's equation was found applicable to butterfat residues, sufficient data was then available for calculation of irrelevant absorption.

The separation of the individual constituents which contribute to the irrelevant absorption of the butterfat residue was attempted. Morton & Stubbs (27) state that if an unsaponifiable residue from a fish-liver oil is taken up in 50% absolute alcohol and is filtered through kieselguhr, much of the irrelevant absorption is lost and relatively pure vitamin A alcohol is recovered in the filtrate. Actually, 50% alcohol precipitates carotene (40), and it was found also, on subsequent filtration through a thick layer of washed kieselguhr on a no. 3 sintered glass funnel, that unprecipitated xanthophyll from butterfat was adsorbed, the resulting filtrate being colourless. Butterfat residues were therefore dissolved in absolute alcohol, an equal volume of water was added and the solution was filtered. The kieselguhr layer was then thoroughly washed with 50% alcohol. The filtrate was finally diluted with water to reduce the alcoholic concentration to 40%, and the vitamin A was quantitatively transferred to petrol ether by shaking with the solvent for 10 min. (40). Readings on the spectrophotometer were taken at 325, 310·5 and 335·5 mμ.

Although there was a certain loss of vitamin A in the process of filtering, etc. (e.g. a 15% loss of added vitamin A), the method proved conclusively that irrelevant absorption by butterfat residues at 325 mμ was not confined to the carotenoids alone. The intensity of absorption of the petrol ether solutions from the filtrates at 310·5 mμ in comparison with the readings at 325 mμ was much greater than that due to pure vitamin A, while the absorption at 335·5 mμ was obviously not significantly increased by the contaminating compound. The linearity of the 'subtraction curve' (26, 27) from a number of these solutions over the range 310·5–335·5 mμ indicated that Morton & Stubbs's equation might be employed to determine irrelevant absorption, and it was found that 8–12% (average 10·0%) of the absorption at 325 mμ after filtration was irrelevant. In view of the failure of Zscheile *et al.* (14) in their endeavour to purify the extract from butterfat residues, no further attempts to eliminate the colourless interfering compounds were made other than by exposing the filtered solutions to ultra-violet light (see later).

The precipitated carotenoid compounds and the adsorbed xanthophyll on the kieselguhr layer were washed through with acetone and, after evaporation of acetone, were dissolved in petrol ether for spectrophotometric readings in the ultra-violet and at 450 mμ. Carotenoids were also isolated from a number of butterfat residues in petrol ether solution by extraction of vitamin A with 90% methyl alcohol (38) and readings were tabled at the same wave-lengths. The average absorption, at 325 mμ, of carotenoids from both these sources was 12% of that at 450 mμ. At 310·5 mμ it was approximately 11% and at 335·5 mμ 13%. These figures vary from one sample to another, due, presumably, to variations in the proportions of carotene isomers present.

The method of Neal *et al.*, viz. irradiation of butterfat residues (25), was repeated except that petrol ether (boiling-point range 55–65° C.) was used as solvent, cyclo-hexane not being available. A number of solutions were irradiated under a Towers ultra-violet lamp for 48 hr. or until the solution was colourless and the density reading at 450 mμ was practically nil. The residual absorption at 325 mμ was of the order of 5% of the original absorption and, judging from experiments on irradiation of carotenoid solutions obtained from butterfat, was due largely to carotenoid breakdown products. Several of the petrol ether solutions of vitamin A from butterfat after the filtration through kieselguhr described above were also irradiated and the spectrophotometric readings were recorded. The residual absorption was very small, and the curve resulting from the subtraction of these

figures from the original absorption figures was no more typical of pure vitamin A than the curve obtained on the solution before irradiation.

With a large number of butterfat residues a correction for irrelevant absorption was attempted by application of Morton & Stubbs's equation. The 'subtraction curve' of Morton & Stubbs under these conditions is the summation of the absorptions due to carotenoids and to non-carotenoid compounds. With some samples the assumption of linearity for both curves and for the combined curve was justified. With other samples, however, the carotenoid curve was non-linear over the range 310·5–335·5 mμ, and the equation was not valid, the calculated correction being obviously in error. It was concluded that the equation was of little assistance as a routine method of correction.

Gillam's method corrects for the absorption due to carotene but not for that due to non-carotene compounds (10). His correction—1/6·5 times or 15·4% of the reading at 450 mμ—appears rather high for carotene alone and may automatically allow for some non-carotene absorption. Zscheile's factor (13) (reading at 324 mμ × 0·78), although it gives a fairly reliable result with many samples, does not take account of variations in the proportion of carotene to vitamin A. As these variations are often considerable from one season of the year to another, the use of such a factor is scarcely justified.

It was concluded that under the experimental conditions described above, approximate corrections for irrelevant absorption, due both to carotenoids and to non-carotenoid compounds, were best calculated as follows:

$$\text{Corrected density reading at } 325\ \text{m}\mu = 9/10\ (A - 12/100B),$$

where A = density reading of the unsaponifiable residue in petrol ether at 325 mμ, B = density reading of the same solution at 450 mμ.

An $E^{1\%}_{1\,\text{cm.}}$ value of 1780 (41, 27) for pure vitamin A alcohol in petrol ether was used for conversion into μg. per g. of butterfat.

Recovery of vitamin A

For recovery experiments weighed amounts of vitamin A concentrates were added to butterfat at 60° C. This was further diluted with melted butterfat before sampling for analysis. An average recovery of 93% of added vitamin A was attained by both the Carr-Price test and by the spectrophotometric method. This figure agrees with the United States Department of Agriculture figure of 93% recovery (1), although their method of saponification and subsequent extraction was slightly different. Vitamin A figures quoted below have not been corrected for the apparent 7% loss.

RESULTS

A comparison of the vitamin A content of a number of butterfat samples, as estimated by (*a*) the Carr-Price test using the tintometer, and (*b*) by the spectrophotometric method, is shown in Table 1.

For samples M 80 and N 80 the tintometer values are 8·3 and 11·1% higher than the spectrophotometric values, but this divergence is probably explained by the very high carotene content of these samples leading to possible errors in the tintometer reading. Otherwise the reasonably good agreement between the two vitamin A values appears to justify the use of the spectrophotometric correction for the experimental conditions described.

Table 1. *Estimation of vitamin A in butterfat samples by the Carr-Price (tintometer) test and by the spectrophotometric method*

Sample	Vitamin A in μg./g. of butterfat	
	Carr-Price test	Spectrophotometric test
A 62	6·7	6·96
B 62	6·5	6·52
M 62	6·4	6·90
N 62	6·4	6·83
F 66	5·6	5·87
A 75	11·0	11·15
B 75	10·1	9·78
A 76	10·5	10·48
B 76	9·0	9·66
H 76	10·9	10·62
I 76	10·1	9·24
M 78	10·3	9·78
N 78	10·0	9·89
M 80	10·8	9·97
N 80	10·3	9·27
A 82	9·7	9·02
A 84	7·6	7·49
M 84	9·4	8·89
H 85	6·8	6·49
M 86	7·5	7·70
N 86	7·6	7·82
Average	8·7	8·59

SUMMARY AND CONCLUSIONS

1. The Lovibond Tintometer has been found more reliable than either the Pulfrich Photometer or the Spekker Absorptiometer for the assessment of the blue colour from the Carr-Price test on butterfat unsaponifiable residues.

2. Using a Beckman Spectrophotometer, vitamin A in butterfat unsaponifiable residues has been estimated also by the intensity of absorption at 325 mμ. From spectrophotometric readings at this wavelength and at 450 mμ the irrelevant absorption, due both to carotenoid and non-carotenoid compounds, may be calculated as follows:

$$\text{Corrected density reading at } 325\ \text{m}\mu = 9/10\ (A - 12/100B),$$

where A = density reading of the unsaponifiable residue in petrol ether at 325 mμ, B = density reading of the same solution at 450 mμ.

3. The Morton & Stubbs equation for irrelevant absorption in fish-liver oils is not generally applicable to butterfat unsaponifiable residues.

4. A comparison of the vitamin A content of butterfat samples by the Carr-Price test and by the direct spectrophotometric method has shown a reasonably good agreement between the two values.

REFERENCES

(1) U.S. Technical Committee in charge of the Nation-wide Survey (1947). *Misc. Publ. U.S. Dep. Agric.* no. 636.

(2) Morton, Lord & Goodwin (1941). *J. Soc. Chem. Ind., Lond.*, **60**, 301.

(3) Oser, Melnick & Pader (1943). *Industr. Engng Chem.* (Anal. ed.), **15**, 724.

(4) Henry, Houston, Kon & Thompson (1944). *J. Dairy Res.* **13**, 329.

(5) Ray, Chand & Rau (1941). *J. Dairy Res.* **12**, 109.

(6) Barnicoat (1947). *J. Dairy Res.* **15**, 80.

(7) Willstaedt & With (1938). *Nord. med. Tidsskr.* **15**, 847. Abstract in *Dairy Sci. Abstr.* (1940). **2**, 174.

(8) VAN BEVER (1938). *Arch. néerl. Physiol.* **23**, 552. Abstract in *Dairy Sci. Abstr.* (1940), **1**, 369.
(9) MORTON & HEILBRON (1930). *Biochem. J.* **24**, 870.
(10) GILLAM (1934). *Biochem. J.* **28**, 79.
(11) SCHRENK, SILKER & KING (1944). *Industr. Engng Chem.* (Anal ed.), **16**, 328.
(12) HUNTER & WILLIAMS (1945). *Biochem. J.* **39**, 5.
(13) ZSCHEILE, NASH, HENRY & GREEN (1944). *Industr. Engng Chem.* (Anal. ed.), **16**, 83.
(14) ZSCHEILE, HENRY, WHITE, NASH, SHREWSBURY & HAUGE (1944). *Industr. Engng Chem.* (Anal. ed.), **16**, 190.
(15) ZECHMEISTER & POLGAR (1943). *J. Amer. chem. Soc.* **65**, 1522.
(16) GILLAM, HEILBRON, MORTON, BISHOP & DRUMMOND (1933). *Biochem. J.* **27**, 878.
(17) GOODWIN & MORTON (1946). *Analyst*, **71**, 15.
(18) BAUMANN & STEENBOCK (1933). *J. biol. Chem.* **101**, 547.
(19) SEMB, BAUMANN & STEENBOCK (1934). *J. biol. Chem.* **107**, 697.
(20) BAUMANN & STEENBOCK (1934). *J. biol. Chem.* **105**, 167.
(21) GILLAM, HENRY, KON & WHITE (1938). *J. Dairy Res.* **9**, 17.
(22) FRAPS & KEMMERER (1938). *Bull. Texas agric. Exp. Sta.* no. 560.
(23) FRAPS & KEMMERER (1943). *J. Ass. off. agric. Chem., Wash.*, **26**, 158.
(24) FRAPS, KEMMERER & MEINKE (1941). *J. Ass. off. agric. Chem., Wash.*, **24**, 731.
(25) NEAL, HAURAND & LUCKMANN (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 150.
(26) MORTON & STUBBS (1946). *Analyst*, **71**, 348.
(27) MORTON & STUBBS (1948). *Biochem. J.* **42**, 195.
(28) EDISBURY (1940). *Analyst*, **65**, 484.
(29) BERL & PETERSON (1943). *J. Nutrit.* **26**, 527.
(30) INNES & BIRCH (1945). *Analyst*, **70**, 304.
(31) CALDWELL & PARRISH (1945). *J. biol. Chem.* **158**, 181.
(32) EMBREE (1947). *Ann. Rev. Biochem.* **16**, 339.
(33) NOTEVARP & WEEDON (1936). *Biochem. J.* **30**, 1705.
(34) BOOTH, KON, DANN & MOORE (1933). *Biochem. J.* **27**, 1189.
(35) STEWART & MCCOLLUM (1938). *J. agric. Sci.* **28**, 428.
(36) MOORE (1930). *Biochem. J.* **24**, 692.
(37) JOHNSON & BAUMANN (1947). *J. biol. Chem.* **169**, 83.
(38) GILLAM & SENIOR (1936). *Biochem. J.* **30**, 1249.
(39) BARTLETT, COTTON, HENRY & KON (1938). *J. Dairy Res.* **9**, 273.
(40) BOYER, PHILLIPS & SMITH (1944). *J. biol. Chem.* **152**, 445.
(41) BAXTER & ROBESON (1942). *J. Amer. chem. Soc.* **64**, 2407, 2411.

(*MS. received for publication* 17 *May* 1949)

637.28

400. FACTORS INFLUENCING THE KEEPING QUALITY OF GHEE*

By D. P. PERSAI† and C. R. BARNICOAT
Massey Agricultural College, Palmerston North, New Zealand

Ghee (dry butterfat) is the most important dairy product of India. Many investigations on ghee have been made in India and elsewhere, particularly in relation to its (*a*) composition, (*b*) vitamin content and (*c*) adulteration by other fats. Problems relating to keeping quality of ghee, however, have not been investigated to any extent, and results given in the literature are rather confusing.

Deterioration of ghee is apparently promoted for the most part by oxidation which detracts both from its nutritive value and flavour. Rancidity (hydrolysis of triglycerides) is also regarded as undesirable, particularly in the higher-quality products.

Of the various factors promoting oxidation in ghee the effect of heat treatment during manufacture has been given considerable attention. Godbole & Sadgopal (5) pointed out that sterilization of ghee by heat increases its subsequent tendency to develop rancidity.‡ Patil & Hammer (11) concluded that butterfat (heated not above 55° C.) and ghee (heated to 110–140° C.) both kept better than the butter from which they were prepared because of the elimination of water and curd. Ritter (13), on the other hand, found butter oil (ghee) prepared by boiling butter to dryness in the presence of curd more resistant to development of oxidized flavour than filtered fat, and Ritter & Nussbaumer (14) noted later that butter oil heated to 100° C. was less susceptible to oxidation than that prepared at 42° C. Mohr (9) concluded that ghee prepared by evaporating at 110° C. was superior to butter oils (filtered at 50 and 90° C.) during 7 months', but not after 11 months' storage. Ewbank & Gould (3) found that heating either butter or butter oil at 127° C. promoted oxidation, while Rafey, Richardson & Henderson (12) observed that butter oil obtained by centrifuging butter heated to 110° C. was more resistant to oxidation than products prepared similarly at lower temperatures. Josephson & Dahle (6) compared ghees prepared by heating (*a*) butter and (*b*) butter oil to 149 and 205° C. for 10 min., and concluded that ghee made by evaporating in the presence of curd was always superior in keeping quality.

Ritter (13) and Rafey *et al.* (12) agreed that improved keeping quality of ghee prepared at higher temperatures is due to transfer of greater amounts of phospholipids from the aqueous to the oil phase. Josephson & Dahle (6), however, found that all ghees prepared from filtered butterfat heated with phospholipids, or with dried skim milk or its components (lactose, casein, etc.) had inferior keeping properties.

As is also the case for butter, acidity is regarded as an important factor in promoting deterioration in ghee. Banerjee & Doctor (1) recommended that in order to ensure good keeping quality, ghee when freshly made should contain not more than 0·15 % acidity (as lactic); an increase in acidity of ghee during storage has also been noted by several workers

* Based on a thesis presented in partial fulfilment of the requirements for the degree of Master of Agricultural Science, 1948, by D. P. Persai.

† Holder of scholarship awarded by J. N. Tata Endowment for higher education of Indians, Bombay.

‡ The term 'rancidity' is used indiscriminately by most workers to mean both rancidity (hydrolysis) and oxidation defects.

(Godbole & Sadgopal(5); Sengupta(16); Narsimhamurty(10); Barnicoat(2)). Patil & Hammer(11) noted that traces of water were deleterious, but Barnicoat(2) concluded that initial acidity rather than moisture was the more important factor, promoting both hydrolytic and oxidative changes in ghee during prolonged storage at low temperatures.

In this paper, results from a series of experiments designed to elucidate the effect of (*a*) nature of the starter, (*b*) method of manufacture, and (*c*) heating conditions on the keeping properties of ghee made from cow butterfat are described.

EXPERIMENTAL

Milk. Fresh milk with cream added to give 8–10% of fat (simulating buffalo milk) was used for these experiments.

Starter. Three cultures (dahi) with differing characteristics* obtained from India,† and two pure cultures of lactobacilli— *L. acidophilus* and *L. bulgaricus*—were used. Cultures of these organisms grown in skim milk at 37° C. attained acidities ranging from 1·0 to 2·5% (as lactic) after about 90 hr., the rate and extent of acid development being characteristic of each organism.

Preparation of ghee. Butter (Makhan) for conversion into ghee was prepared according to methods recommended in the Indian Dairy Department's Report for 1940–1, i.e. inoculating milk cooled to 100° F. after boiling with 5–10% starter, incubating at room temperature for 24–48 hr. (until the milk clotted) and churning at 60–80° F. with, if necessary, addition of 5–10% water. Warm breakwater was also added and cold water at the granule stage. The churnings were made in a well-tinned 1 gal. 'Alfa-Laval' churn.

For conversion of Makhan into ghee, it was heated for various periods at temperatures ranging from 80–150° C. over a hot-plate controlled by a 'Sunvic' energy controller. The ghee was finally filtered through several layers of coarse filter-paper.

In the early stages other methods of manufacture were tried (e.g. French(4)), but as none of these yielded products in any way superior to those derived from the foregoing method, no further work along these lines was done.

Keeping quality tests. Keeping qualities were assessed from the times required by samples to reach the end of their oxidation-induction periods, as found from graphical data. This choice was not arbitrary because at this stage bleaching or partial bleaching was evident (indicating loss of the vitamin A precursor, carotene), and the flavour was also impaired.

Keeping tests were made under two conditions: (*a*) a practical test at 38° C. ('Indian summer temperature') for 4–8 months, and (*b*) an accelerated test at 100° C. (Swift oxidation tester(18)).

Analytical methods. (*a*) *oxidation tests*: (i) peroxide value (Lea(7)), (ii) fat aldehyde value (Schibsted(15)); (iii) carotenoid-pigment value—an arbitrary measurement in a Spekker absorptiometer of a petroleum ether solution of fat. The relative rates of oxidation, as found by these three methods, agreed quite well, but as the peroxide test was the simplest to carry out it was used as a measure of oxidation in this investigation.

(*b*) *acidity*, titration of boiling neutralized alcohol extract, and (*c*) *moisture*, drying at 100–110° C. (as proposed by Sunawala & Kothavalla(17)), gave erratic results through

* Culture (*a*) small slender rods—grew slowly in milk, (*b*) middle-sized rods—grew well in milk, and (*c*) diplococci—grew well in milk.

† Through the courtesy of Dr Zal. R. Kothavalla, Dairy Development Adviser, Government of India.

samples tending to increase in weight during the first 3–36 hr., therefore a rapid 'factory test' (as used by butter-makers) was employed, duplicates varying only by ±0·01% moisture.

General. As the investigation mainly consisted of comparisons of series of fats, a control sample of butterfat (heated not above 80° C.) was included in each series in order to check the quality of the original butterfat.

RESULTS

(1) *Effect of different starter cultures on the keeping quality of ghees*

Preliminary experiments indicated that ghees made with *acidophilus* cultures had better keeping properties than those made with *bulgaricus*. The latter culture attained a higher final acidity (2·5%) than *acidophilus* (1·4%), which may have contributed to the differences in the keeping qualities of the ghee. Ghees were therefore made from milks ripened with the five cultures to the same acidity (0·9% (as lactic)), different cultures taking different times to attain this state. Results are shown in Table 1.

Table 1. *Effect of different starter cultures on the keeping quality of ghees*

Starter	Cooking temperature (° C.)	Fat oxidation induction time: Accelerated test at 100° C. (hr.)	Fat oxidation induction time: Holding test at 38° C. (weeks)	Acidity (as oleic %) (holding test): Initial	Acidity (as oleic %) (holding test): Final
Dahi (*a*)	110	18	20	0·68	0·90
	130	30	24	0·68	0·91
Dahi (*b*)	110	18	20	0·73	0·84
	130	44	28	0·73	0·88
Dahi (*c*)	110	22	24	0·68	0·73
	130	46	28	0·68	0·76
L. acidophilus	110	26	24	0·68	0·85
	130	30	24	0·73	0·85
L. bulgaricus	110	18	20	0·73	0·85
	130	56	32	0·73	0·76

From Table 1 it is seen that:

(*a*) There were considerable variations in the keeping properties of ghees made from milk ripened to the same acidity with different starter cultures, as shown by both accelerated tests at 100° and holding tests at 38° C. (The most notable differences were in their flavours.)

(*b*) Ghees prepared by heating to 130° C. were considerably better in keeping quality than similar products heated to 110° C.

(*c*) Acidities increased slightly during storage.

It was also noted that samples had bleached at the end of the induction period, but not to the same extent.

(2) *Effect of differences in initial acidity on keeping quality of ghees*

Milk was inoculated with 10% of a culture of *L. bulgaricus* (the most vigorous acid-producer), and four samples of 1·1, 1·7, 2·2 and 2·5% acidity were churned and made into ghee by evaporating to 110 and 130° C. The results are shown in Table 2.

Table 2. *Effect of variations in initial acidity on keeping quality of ghees*

Treatment	Cooking temperature (° C.)	Fat oxidation induction time: Accelerated test at 100° C. (hr.)	Fat oxidation induction time: Holding test at 38° C. (weeks)	Acidity (as oleic %) (holding test): Initial	Acidity (as oleic %) (holding test): Final	% moisture
Control (unripened)	80	12	16	0·17	0·45	0·04
	130	30	20	0·17	0·34	0·19
Ripened to 1·1% acidity	110	12	16	0·34	0·56	0·46
	130	42	28	0·51	0·61	0·27
Ripened to 1·7% acidity	110	18	16	0·68	0·86	0·23
	130	30	24	0·68	0·78	0·19
Ripened to 2·2% acidity	110	18	16	0·90	1·21	0·24
	130	42	28	0·90	1·35	0·53
Ripened to 2·5% acidity	110	18	16	0·50	0·56	0·19
	130	18	20	0·51	0·56	0·29

From Table 2 it is seen that:

(*a*) There was no relationship between acidity of milk at churning, or initial acidity of ghee, and keeping quality in either the accelerated or holding test.

(*b*) The ratio of churning acidity to ghee acidity varied widely, i.e. from about 7 to 12:1.

(*c*) Increases in acidity during storage for 28 weeks at 38° C. were irregular and in no way related to keeping properties of the products.

(*d*) There was no correlation between moisture content of ghee and keeping quality.

(3) *Relation between keeping quality of ghees and variations in heat treatment during the cooking process*

The foregoing results clearly show that ghees heated to 80 or 110° C. have consistently poorer keeping qualities than ghees made from other portions of the butter heated to 130° C. Experiments were accordingly made in order to determine the nature of the substances responsible for these differences. Milk ripened with *L. bulgaricus* to 1·2% acidity was churned and the butter divided into two lots. The first was heated at 80° C. for 30 min., filtered and divided into portions which were heated to 110, 130 and 150° C. momentarily (i.e. without contact with curd). The other lot was heated under similar conditions, but according to normal ghee-making procedure (i.e. in contact with curd). Results from these experiments are shown in Table 3.

Table 3. *Relation between keeping quality of ghees and variations in heat during cooking process*

Treatment	Temperature (° C.)	Fat oxidation induction time: Accelerated test at 100° C. (hr.)	Fat oxidation induction time: Holding test at 38° C. (weeks)	Acidity (as oleic %) (holding test): Initial	Acidity (as oleic %) (holding test): Final	% moisture
Control, 30 min.	80	18	12	0·11	0·23	0·23
Heated momentarily to final temperature without contact with curd	110	16	12	0·11	0·28	0·30
	130	12	8	0·17	0·28	0·17
	150	8	8	0·17	0·28	0·22
Control, 30 min.	80	16	12	0·17	0·23	0·22
Heated momentarily to final temperature in contact with curd	110	20	14	0·17	0·23	0·19
	130	24	16	0·23	0·33	0·37
	150	36	Exceeds 20 weeks	0·23	—	0·35

Results in Tables 1 and 2 emphasized the major importance of the cooking process in determining keeping properties of ghee, and the results in Table 3 indicate that while heating pure butterfat to higher temperatures reduced its storage life, heating in contact with curd (as in the normal ghee-making process) gave products with storage life increasing more or less in proportion to the cooking temperature.

(4) *Effect of rate of cooking and amount of stirring on keeping quality of ghee*

Experiments were made in order to study the influence on the quality of the product of (*a*) rate of heating, i.e. by comparing the products made under slow heating conditions (100 min. to attain final temperature) and rapid heating (25 min.), and (*b*) amount of stirring during cooking, i.e. by comparing the products made with continuous stirring with those made in the normal way (stirred three or four times).

Results showed that these factors were of no importance to ghees made at a temperature of 110° C., but excessive stirring and prolonged (i.e. slow) heating had very damaging effects on ghee evaporated at 130° C.

(5) *Experiments to determine the nature of the antioxidants incorporated in ghee during cooking*

In order to determine the nature of the substances responsible for the antioxidant properties recorded in Table 3, portions of butterfat (filtered at 80° C.) were heated with 1% lactose, casein, skim-milk powder or fresh egg-yolk to 110 and to 130° C., and filtered. Results for oxidation tests with these fats are shown in Table 4.

Table 4. *Experiments to determine nature of antioxidants derived from curd during cooking*

Substance added	Temperature (° C.)	Fat oxidation induction time by accelerated test at 100° C. (hr.)
Nothing (control)	80	12
Nothing (control)	110	12
1% lactose	110	12
1% (lactic) casein	110	12
1% skim-milk powder	110	12
1% egg-yolk	110	18
Nothing (control)	130	6
1% lactose	130	4
1% (lactic) casein	130	6
1% skim-milk powder	130	6
1% egg yolk	130	12

In Table 4 it is seen that only egg-yolk—a source of phospholipids—promoted increase in induction periods. The other substances—lactose, casein, and skim-milk powder—proved valueless as sources of antioxidants.

While these experiments support the theory that phospholipids extracted from curd during the cooking of ghee play an important part in enhancing its storage life, it is possible that other antioxidants may be formed from the curd by heating. The fact that the storage life of ghee increased progressively with the final cooking temperature attained (see Table 3) suggests that in addition to favouring the incorporation of phospholipids, high temperatures may also have converted other substances into antioxidants. Lea (8),

for example, has shown that sulphydryl compounds arising from heat-denatured proteins act as antioxidants in skim-milk powders.

Several series of experiments were carried out in an attempt to detect the incorporation of antioxidants of other than phospholipids from heated curd. For example, milk solids-not-fat, prepared at low temperatures (freeze dried) and at 80, 100 and 130° C., were mixed with butterfat at 80° C. (momentarily or for 30 min.) in order that the fat might extract any preformed antioxidants. Oxidation tests of the filtered fats indicated, however, that antioxidants were not formed in curd heated to high temperatures out of contact with butterfat.

(6) *Relationship between storage life of ghee at* 38° C. (*Indian summer temperature*) *and its rate of oxidation at* 100° C. (*accelerated test*)

The temperature coefficient (Q_{10}) for peroxide formation of butterfat held in the dark at temperatures between 38 and 100° C. was 2·0.

The relationship between rates of oxidation of twenty-eight samples of butterfat (ghee) at 38 and 100° C. (Swift oxidation tester(18)) is shown in Fig. 1.

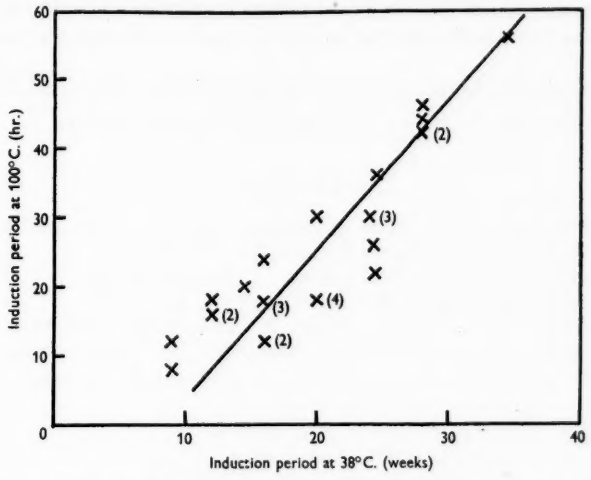


Fig. 1. Graph showing relationship between induction periods for oxidation of ghees. (y axis = time in hours at 100° C.: x axis = time in weeks at 38° C.) Brackets indicate number of observations falling at any one point.

Correlation (r) = 0·892 (H.S.). From these results the following equation was calculated:

$$\text{Storage life of ghee (weeks)} = 8{\cdot}5 + 0{\cdot}46X,$$

where X = time in hours for sample to reach end of induction period (peroxide value) at 100° C. (Standard error of estimate = 3·1 weeks). These results indicate that the storage life of ghee can be predicted from accelerated tests with a reasonable degree of accuracy.*

* This equation is probably applicable only to the particular conditions in these experiments.

SUMMARY

Of the four main factors commonly regarded as influencing keeping properties of ghee, viz. moisture content, acidity, heat treatment and type of bacterial culture used in souring, the heat treatment was found to be the most important.

In general, the higher the final temperature (110° C. and higher) attained during evaporation, the better were the keeping properties of the product, providing that the butter had been heated in contact with curd, boiled as rapidly as possible, and given a minimum amount of stirring.

The improved keeping properties conferred on ghee heated to 110–150° C. were promoted by antioxidants (apparently phospholipids) extracted from the curd during cooking.

Initial acidity and moisture contents had no effect on storage life of ghee at 38° C., and the practice of grading ghee according to its acidity would appear to be of questionable value. Souring of milk to give 2·5–3·0% lactic acid and the accompanying desirable flavour did not detract from the keeping quality of the ghee made from it.

The type of bacterial culture used for souring had only slight influence on keeping quality of ghee, but a marked influence on flavour.

Storage life of ghee at 38° C. ('Indian summer temperature') could be predicted with fair accuracy from its rate of oxidation at 100° C. in a 'Swift oxidation tester'.

The writers are grateful to members of the staffs of Massey Agricultural College and the the Dairy Research Institute (N.Z.) for helpful criticism and advice.

REFERENCES

(1) Banerjee, B. N. & Doctor, N. S. (1938). *Agric. Live-Stk India* **8**, 158.
(2) Barnicoat, C. R. (1945). *N.Z. J. Sci. Tech.* **27**, 309.
(3) Ewbank, F. C. & Gould, I. A. (1943). *J. Dairy Sci.* **26**, 409.
(4) French, M. H. (1938). *Bull. imp. Inst., Lond.*, **36**, 349.
(5) Godbole, N. N. & Sadgopal (1936). *Z. Untersuch. Lebensmitt.* **72**, 35.
(6) Josephson, D. V. & Dahle, C. D. (1946). *Dairy Sci. Abstr.* **8** (1), 49.
(7) Lea, C. H. (1938). *F.I.B. Spec. Rep.* no. 46 (Dep. S.I.R. England).
(8) Lea, C. H. (1946). *Analyst*, **71**, 227.
(9) Mohr, W. (1939). *Molkereiztg, Berl.*, **53**, 2304.
(10) Narsimhamurty, G. (1941). *Analyst*, **66**, 98.
(11) Patil, V. H. & Hammer, B. W. (1928). *J. Dairy Sci.* **11**, 143.
(12) Rafey, E. L., Richardson, G. A. & Henderson, J. L. (1944). *J. Dairy Sci.* **27**, 807.
(13) Ritter, W. (1937). *Proc. World's Dairy Congr.* **2**, 156.
(14) Ritter, W. & Nussbaumer, T. H. S. (1939). *Chem. Abstr.* **33**, 8837, 9467.
(15) Schibsted, H. (1937). *Industr. Engng Chem.* (Anal. ed.) **4**, 204.
(16) Sengupta, P. N. (1943). *J. Indian Chem. Soc.* **6**, 158.
(17) Sunawala, S. D. & Kothavalla, Z. R. (1935). *Agric. Live-Stk India*, **5**, 480.
(18) Wheeler, D. H. (1932). *Oil & Soap*, **9**, 89.

(*MS. received for publication* 23 *June* 1949)

637.325

401. CHEESE GRADING. MECHANICAL PROPERTIES COMPARED WITH QUALITY ASSESSED SUBJECTIVELY

By R. HARPER and M. BARON
National Institute for Research in Dairying, University of Reading

(With 2 Figures)

During the course of a study of the mechanical properties of Cheshire cheese extending over a period of about 2½ months, ratings were obtained for the main qualitative attributes from a small panel of judges. The function of these ratings was to form a link between instrumental measurements of the mechanical properties and the subjective assessment of the firmness, springiness, crumbliness and overall quality of the cheese. This aspect of the data will be discussed elsewhere (1). The ratings obtained offer an opportunity for studying individual differences on the part of the judges and for assessing the relative importance and effectiveness of the separate attributes in predicting the final development of quality.

Between five and eight cheese were rated on a single day by a group of at least three individuals. The same cheese were rated both when 2 weeks old and when 2 months old, forming in each case the same test batches. Unfortunately, the groups of judges were not identical throughout the study. When rating the cheese at 2 weeks, three groups of individuals were involved, but one judge remained common to all groups. A single group of judges rated the entire set of cheese aged 2 months. Ratings for firmness and springiness were made on the whole cheese (i.e. on the outside, through the rind); ratings for firmness, springiness, crumbliness and quality were made on a boring or plug from each cheese. A seven-point rating scale was used for firmness and quality, since it was expected that only these could be assessed with any fine degree of discrimination. Five points were used for rating the other attributes, but these will not be discussed in the present paper. Comments on flavour and any other striking characteristics were recorded. One of the judges in each group was professionally connected with cheese-making, while the other judges were working on the mechanical testing of the cheese. One of these non-professional judges had spent several years, intermittently, in cheese factories and had previously made extensive judgements on the firmness, flavour and quality of cheese.

Differences in ratings may arise from several causes. There may be real differences between cheese, or there may be a systematic bias which differs from one judge to another. There are likely to be random errors resulting from the combined effect of complex causes such as variations in the general health of the judges, distractions, inherent fluctuation of judgement, interest factors and order of sampling the cheese. As the present study is essentially preliminary, it is not intended to make a detailed statistical analysis of the data, but rather to present the information, as far as possible, in a graphical form. Where differences between cheese are being studied, it has been considered best simply to average the ratings for all the judges, although some error is likely to be introduced when the composition of the group of judges changes. To correct for this would involve refinements of technique hardly justifiable at the present stage. Where differences between individuals are being considered, ratings on all the cheese forming a single batch have

been averaged for each judge. In view of the extensiveness of the data, the relationship between the various aspects of the cheese are best shown by averaging groups of ratings. By doing so the accuracy of prediction appears more precise than it would for a single judgement, but the underlying factors common to the various sets of ratings are brought out effectively.

DIFFERENCES BETWEEN INDIVIDUALS

The discussion of individual differences will be confined to judgements made by the same group of judges on the entire set of cheese when 2 months old. Since the individual discrimination of springiness and crumbliness with the present set of cheese does not appear to be very marked, only firmness and quality will be considered. It may, however, be noted that the cheese-maker who was one of the judges was unable to make discrimination between the cheese for springiness as distinct from firmness, and left the appropriate section of the record form blank. The ratings, averaged for each batch of cheese, are given in Fig. 1 for each of three individuals. Differences in the assessment of quality and firmness will be discussed separately.

In spite of having been given the same instructions, different individuals view the same cheese as belonging to different quality levels, although there is considerable agreement on the differences between many of the groups. The most marked difference in general level is between *A* (the cheese-maker) and *B*, amounting to an average deviation of 1·1 units on the seven-point scale* and representing a higher judgement for overall quality by the cheese-maker. This is, presumably, a perfectly understandable human bias, the existence of which is confirmed by the fact that even when making serious criticisms of a cheese such as, 'Mouldy, off flavour, sour', or 'Off flavour, badly tainted', only a rating 4 was used. (Out of 200 cheese only one was rated as high as 5 by the cheese-maker and none higher than 5.)

The most striking differences in opinion occur in batches 11–16 which are seriously down-graded by *B* even after allowing for the differences in average levels of rating.

Analysis of the comments made on these particular batches by *B* are so definite and critical as to leave no doubt as to the adverse impression derived. The number of cheese in these six batches receiving pronounced critical comments by the three judges were

A	*B*	*C*
6	15	5

Three times more adverse comments were made by *B* than by the other two judges.

In view of these differences in opinion on taste and quality, it is surprising to find such a high level of agreement between the average rating for firmness and for quality, which is discussed below. However, for studies of the present type these differences of opinion make it desirable that a larger panel of judges should be used. Furthermore, if there is to be available an internal measure of the accuracy of a single individual, then judgements must be made at least twice on the same cheese, taking care to eliminate any recollection of the previous rating. Where commerial grading is concerned, it is likely that the idiosyncrasies of the grader also play a part in determining his judgement. Presumably, the grader may be trained to represent public taste when, in times of plenty, this is able to express itself effectively.

The firmness ratings on the cheese, aged 2 months, show certain systematic trends for at least one individual (*B*). If the cheese-maker is taken as 'standard' at the beginning

* On the quality scale 1 denotes excellent and 7 very poor.

of the judging at 2 months, *B* finds the cheese much firmer than the experienced judge but, as the experiment progresses, the difference of opinion is reduced. It is perhaps of interest to note that *C*, although not experienced in judging the firmness of cheese, had taken part in a large number of experiments handling cheese-like materials and that she shows no marked systematic changes in the deviation between her ratings for firmness and those of the cheese-maker.

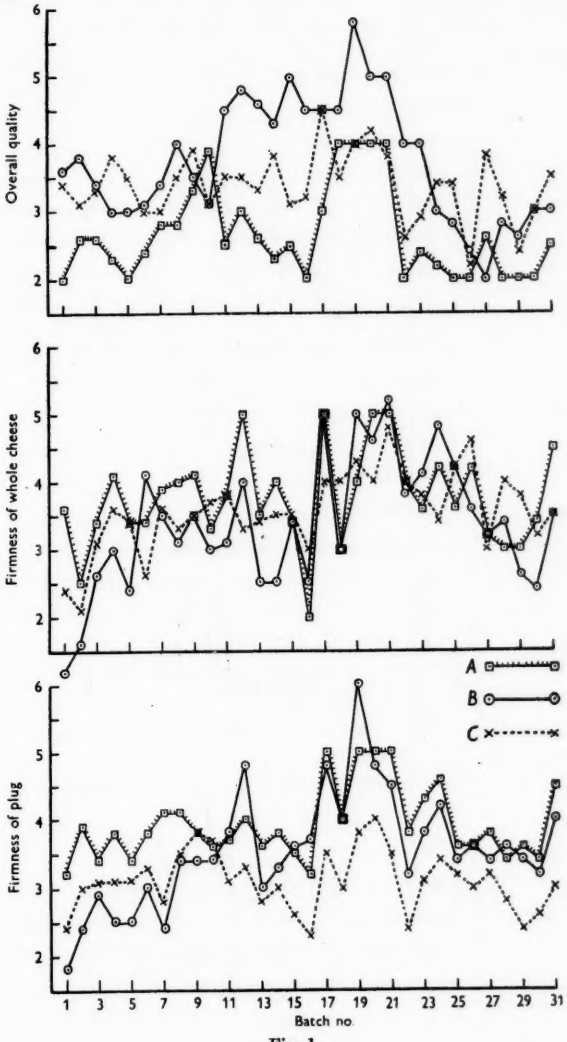


Fig. 1

GENERAL RELATIONSHIPS

In Fig. 2 the average ratings for all individuals on the batches of cheese made on separate days are given. In particular, a marked relationship between firmness and quality is evident. The ratings on individual cheese have been analysed, and it has been found that

of the eleven cheese estimated to have the poorest quality when 2 months old (mean rating greater than 4·4 on the present scale), nine of these could have been screened off simply on the basis of their firmness ratings at 14 days (i.e. when, under present conditions, they

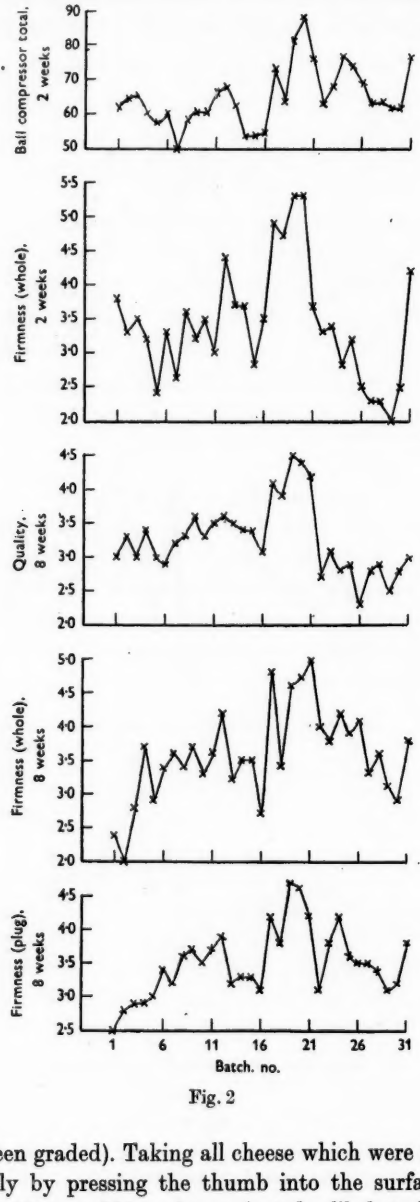


Fig. 2

would normally have been graded). Taking all cheese which were rated more than 4·6 for firmness,* judged simply by pressing the thumb into the surface of the cheese, then twenty-eight cheese out of 211 would have been selected as likely to develop unsatisfactorily. Of these, nine would in fact have done so, out of the total of eleven ultimately seriously

* On the firmness scale 1 denotes very firm, 7 very soft.

down-graded. The actual quality level for rejection is arbitrary, but a rejection rate of approximately 5% is not considered unnecessarily lenient.*

It should, of course, be remembered that the ratings on the 211 cheese were carried out in small groups of about five to eight cheese on any one day. In commercial grading the grader may be faced with several hundreds of cheese as a single morning's work. He can therefore neither taste all the cheese nor can he be expected to assess them all for firmness. If he did, his judgements in bulk would become subject to serious deterioration through fatigue and adaptation. This certainly applies to tasting and may also apply to judgements of firmness although reliable objective evidence is not available on this point. However, a modern version of the Ball Compressor described by Caffyn & Baron (2), originally proposed by Scott Blair & Coppen (3) gives an instrumental measurement correlating highly with judgements of firmness (see Fig. 2). This instrument could be used by an unskilled person after a little training, to test *all the cheese*. The best opportunity for making such measurements would be when the cheese are being removed from the shelves for weighing purposes, when two measurements might be obtained from each end of the cheese. It is not claimed that prediction of quality by firmness measured instrumentally or by the thumb will be infallible. However, if a substantial proportion of cheese which will develop unsatisfactorily can be screened off by such methods, and if the number which have to be examined in detail and therefore subject to minor injury by boring can be reduced from 211 to twenty-eight, the cumulative effect of the adoption of these new methods would be commercially profitable and important.

SUMMARY

An experiment is described in which ratings on some 200 cheese made on 31 different days were rated for firmness, quality, springiness and crumbliness, both when 2 weeks and 8 weeks old. Some of the relationships between the ratings are discussed, in particular the ratings for firmness and quality. By means of the firmness ratings made on the cheese at 2 weeks, twenty-eight cheese out of 211 could have been screened off, and of these twenty-eight, nine were subsequently seriously down-graded for quality. Only two cheese which were not screened off developed unsatisfactorily to the same degree. Individual differences between judges are noted, especially with regard to quality. The cheese-maker considers the cheese to be about 1 point higher in quality (on a 7-point scale) than the remaining judges. One judge who criticizes adversely about three times as many cheese as the remainder of the judges also down-grades about six batches of cheese which are not down-graded by the remaining judges.

The writers are indebted to the Directors of United Dairies Ltd. for placing facilities at their disposal at the Ellesmere Creamery, and to the Manager and Staff of that Creamery for their co-operation during the study.

They also wish to acknowledge grants from the Agricultural Research Council covering the period of this investigation.

REFERENCES

(1) In process of publication.

(2) CAFFYN, J. E. & BARON, M. (1947). *Dairyman*, **64**, 345.

(3) SCOTT BLAIR, G. W. & COPPEN, F. M. V. (1941). *J. Dairy Res.* **12**, 44.

* Some of the cheese were made during hot weather from salvaged milk.

(*MS. received for publication* 24 *February* 1949)

637.333:576-809.6

402. PHAGE-CARRYING CULTURES AS CHEESE STARTERS

By G. J. E. HUNTER and H. R. WHITEHEAD
Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 1 Figure)

The practical problems involved in the maintenance of cheese starters in an active state in commercial dairy factories have been described in previous papers from this Institute (1, 2, 3). It has been shown that infection of the mother and bulk cultures is most liable to occur as a result of the presence in the factory surroundings of a heavy concentration of air-borne phage-containing whey droplets. Various methods suggested and used for the protection of starter cultures from this infection are based on the two principles:

(*a*) Removal of the starter during preparation to a more lightly infected atmosphere (a non-infected atmosphere if possible).

(*b*) Sterilization of the air which gains direct access to the culture, by filtration, heat treatment or ultra-violet light treatment.

We are not here concerned with other forms of phage infection which may interfere with the cheese-making process but merely with infection of the starter culture itself.

In the earlier years of our investigations the possibility of preventing starter failures by the use of phage-resistant cultures was visualized (4). It was soon found, however, that cultures which had been rendered resistant to a given phage were invariably soon attacked by other phages when used under commercial dairying conditions. This line of investigation was abandoned and attention was concentrated on methods for the protection of sensitive cultures from phage infection. These methods are now applied almost universally in New Zealand cheese factories.

Recently, however, it was observed (5) that some streptococcal strains could grow in association with certain phage races, and that the presence of a phage conferred on a culture a measure of protection against attack by other phages. This observation raised anew the question as to whether it might not be possible to give 'internal' protection to a commercial starter and thus render unnecessary the use of devices designed to give protection against air-borne phage. The fundamental characteristics of the phage-organism association have already been reported (5). The present paper deals with attempts to make use of phage-carrying cultures in cheese-making practice.

ISOLATION OF THE FIRST PHAGE-CARRYING CULTURE

The phenomenon of associative growth of *Streptococcus* and phage was first observed by chance when, during a search for strains of streptococci suitable for use as cheese starters, a culture OP was isolated from a 'mixed' starter in use at a commercial cheese factory. Investigation of the purified culture led to the conclusion that it was a partially resistant form of culture HP, a stock strain which has been maintained in this laboratory and supplied regularly to commercial cheese factories for more than 12 years. Phage race 2 which most frequently attacks HP in commercial practice (6) failed to lyse OP in milk culture at any temperature between 20 and 37° C., but on an agar surface spread with OP a few plaques

appeared where a drop of a high-titre preparation of the phage was placed. When a trace of phage 2 was added to a milk culture of OP, and the culture was then subcultured daily in milk in the usual way, it was found that although the culture continued to clot normally, clear whey obtained by centrifuging the clotted culture regularly contained phage 2 (active against HP) in a titre of 10^{-4} or 10^{-5} as estimated by the drop method on agar. This 'phage-carrying' culture was designated OP + 2.

Use of the original culture OP as a cheese starter resulted in the appearance in the whey of a phage race 36 to which the culture was sensitive. This accorded with our previous experience that resistant or semi-resistant strains are soon infected under commercial conditions with other phages to which they are sensitive. Other phage races (10, 34 and 37) isolated from various sources were also active against OP. Hunter (5) showed that whereas OP was lysed by phages 36, 10, 34 and 37, culture OP + 2 (the phage-carrying culture) was not lysed by race 36 because, apparently, its action was blocked by the presence of the related phage 2. The action of unrelated phages 10, 34 and 37 was not blocked by the presence of phage 2. The protective action of phage 2 when associated with OP was further demonstrated in 'vitality' tests (6) in which 150 ml. quantities of pasteurized milk were inoculated with 1·5 ml. of the clotted culture and were subsequently renneted and formed into cheese curd. The results in Table 1 show how phage-carrying culture OP + 2 formed acid at a normal rate even when the curd was contaminated with phage race 36.

Table 1. *Acid production by OP cultures in cheese curd contaminated with phage race* 36

Acidity as parts per thousand lactic acid

Culture	Test milk	1st titration	2nd titration	Increase
OP	Control	3·0	6·6	3·6
OP	+0·1 ml. phage 36	2·0	2·8	0·8
OP + 2	Control	2·9	6·6	3·7
OP + 2	+0·1 ml. phage 36	3·3	7·2	3·9

TRIALS OF CULTURE OP + 2 IN THE CHEESE VAT

The three cultures HP, OP and OP + 2, prepared with every care to avoid contamination with extraneous phage, were used separately daily in three cheese vats in the experimental factory. The whey from the vats was skimmed in a separator in the cheese-making room. In order to determine whether air-borne phage was present three beakers containing sterilized skim milk were exposed for 1 hr. each day near the separator while it was running. The beakers were then covered with Petri dish lids, and were inoculated with cultures HP, OP and OP + 2 respectively and incubated at 22° C. overnight. Each day three fresh batches of sterilized milk were exposed to air-borne contamination in this way, and each beaker was inoculated from the corresponding beaker of the previous day. The procedure thus simulated the transference of starters from bulk culture to bulk culture under commercial conditions and with no precautions against contamination with air-borne phage. The results were clear-cut. The milk inoculated with HP failed to clot after the first exposure, indicating the presence of phage 2. The HP beaker repeatedly failed to clot when it was inoculated freshly each day from a laboratory culture. This was to be expected, since starter HP was in regular use in the cheese factory. The beaker of milk inoculated with OP clotted on the first and second days but failed to clot on the third day, indicating a lower but possibly increasing concentration in the air of a phage capable of lysing OP.

The beaker inoculated with OP+2 and transferred daily continued to clot normally for the whole week of the experiment. As a further extension of the trial culture OP+2 was then prepared as a bulk starter daily in an open vessel near the separator and was used in a cheese vat. Tests for the presence of air-borne phage showed the regular presence of a phage capable of attacking OP.

Although these results were quite promising from the practical point of view, it was evident that there were some disadvantages associated with the use of OP+2 as a cheese starter:

(*a*) The culture did not retain its associated phage indefinitely. In some preparations the phage persisted in the culture for periods of up to 3 months, but it was liable at any time to die out in a few days, thus exposing OP to attack by phage race 36. Constant checks in the laboratory were necessary when culture OP+2 was in use as a starter under conditions where no protection against air-borne phage was afforded.

(*b*) The protection conferred by phage 2 was not effective against all phage races capable of attacking OP. We do not yet clearly understand what factor determines which of several possible phages shall establish itself in a given cheese vat. Perhaps it is a matter of chance. Thus although in our experiments no failures occurred over quite a long period with cultures of OP+2 there was no guarantee that on some other occasion or in some other factory another phage race, capable of attacking OP and not blocked by phage 2, would not appear in the cheese whey.

PREPARATION OF A 'PHAGE-CARRYING' CULTURE FROM HP

In an attempt to overcome the second of the two disadvantages visualized in connexion with OP+2, phage-carrying cultures were prepared from culture HP by preparation of resistant forms in the laboratory. Hunter (5) had already shown that some resistant forms of HP were capable of associative growth with phage 2, and that they were then protected against the action of a greater variety of phage races than was culture OP+2. A series of cultures HP/2+2* were therefore tried as starters in the cheese vat under conditions which would lead to failure with normal single strain cultures.

A completely resistant (non phage-carrying) culture HP/2 was first used as a starter in a vat with the addition to the cheese milk of a particularly active phage (race 34). This phage has a wide range of action among the various strains related to HP. A typical phage failure occurred in the vat during the course of cheese manufacture, and passage of the whey through the separator resulted in the widespread dissemination of phage 34 throughout the atmosphere. On subsequent days the phage-carrying culture HP/2+2 was prepared as a bulk starter in an open vessel in the cheese room and was used in the manufacture of cheese. The starter behaved quite satisfactorily in spite of the presence of phages 2 and 34 in the atmosphere, as was demonstrated in the usual manner by exposure of sterilized milk and subsequent inoculation of HP and HP/2. In this way cultures of HP/2+2 were shown to be unaffected both in the laboratory and in the cheese factory by a series of phage races capable of attacking HP/2. Thus the phage-carrying cultures prepared from HP had an advantage over the OP cultures in that they were protected against a wider range of phage races. They still had the disadvantage, however, that the persistence of phage in its association with the organism was somewhat uncertain. Furthermore, the phage-carrying cultures prepared from HP had an additional slight disadvantage in that

* This notation signifies an HP culture partially resistant to phage 2 and living in association with phage 2. For more detail see Hunter (5).

they were less vigorous in acid production than HP and JP. It was necessary to use 2¼–2½% of HP/2+2 in the cheese milk to get the result given by 1½–1¾% of HP. Phage-resistant cultures are usually less active than the parent strains, and there are indications that with phage-carrying strains the greater the range of phage races against which a culture is protected the less vigorous is the culture as an acid producer.

TRIALS OF PHAGE-CARRYING CULTURES IN COMMERCIAL CHEESE FACTORIES

In the Canterbury district of the South Island of New Zealand there is a group of ten small cheese factories which have not yet been fitted with starter equipment designed to give protection against air-borne phage infection. An opportunity was thus offered for the trial of phage-carrying cultures under conditions where it has been proved by experience that normal single strain starters would quickly fail. In collaboration with Mr G. A. Challies, the Department of Agriculture Instructor for the district, a trial was conducted in several cheese factories over parts of two dairying seasons. Cultures of HP/2+2 were supplied regularly from the Institute and were renewed at 3-weekly intervals. In the factories the starters were maintained as mother cultures in cotton-wool-plugged flasks and were subcultured with aseptic technique. The bulk starters were prepared in open cans subject to air-borne contamination.

The results for several months were similar to those obtained at the Institute experimental factory. The starters behaved quite normally and cheese of good quality was produced. The cheese was much closer in texture than that previously made at these particular factories. This was in accord with expectations, and was the result of the change from a mixed commercial starter to a single-strain culture. This first trial was conducted during the closing months of one season. At the beginning of the next season the trial was recommenced at first with satisfactory results. After about 6 weeks, however, some managers reported a reduction in the activity of the starters resulting in 'slow' (but not 'dead') vats. Samples of starter and of whey from the slow vats were examined, but at first nothing abnormal could be detected. The starter still contained the associated phage 2 and was normal in activity. No lytic agent against HP/2+2 could be found in the whey by trial either on an agar surface or in a liquid medium, but when the starter was sown into milk samples with and without additions of the Seitz-filtered whey and the acid development was followed by titration, it was found that there was present in the whey some agent which depressed acid production. Further investigation showed that the effect of whey from the slow vats was definitely due to the presence of a phage. Fresh preparations of HP/2+2 made by plating out older preparations and re-adding phage 2 were frankly attacked by the phage in the 'slow' whey both on the surface of agar and in liquid medium, but continued daily subculture rendered HP/2+2 gradually less sensitive to the action. This decreasing sensitivity showed itself first by an absence of plaque formation on agar plates and a resistance against complete lysis in milk cultures. At this stage the phage in the whey still had a depressing effect on the growth of the culture and hence on acid production. On continued subculturing the culture finally reached a stage where it was quite unaffected by the new phage. The practice which had been adopted in our commercial trial of renewing the factory cultures frequently with fresh preparations of HP/2+2 (in order to make sure that the protective phage was constantly present) had actually rendered the cultures more susceptible to attack by this new phage. The effect of the 'age' of a phage-carrying culture on its sensitivity is illustrated in Fig. 1, where acid production by a HP/2+2 culture with (broken line) and without (full line) additions of

filtered whey from the slow vats is graphed. The four graphs show how the rate of acid production changed as the culture 'aged'. The effect of whey additions in depressing acid formation by the cultures decreased progressively with 'age' of the culture, the term 'age' being used in the sense of 'time elapsed since the culture was started from a colony picked from a plate'. All preparations of HP/2+2 do not show the same rate of change in sensitivity. In the case to which the graphs refer the culture became quite insensitive to the phage present in the filtered whey in 4 weeks from the time of isolation of the culture. All other cultures showed an equivalent change, sometimes in shorter and sometimes in longer periods. The cultures throughout these 'ageing' periods were quite insensitive to

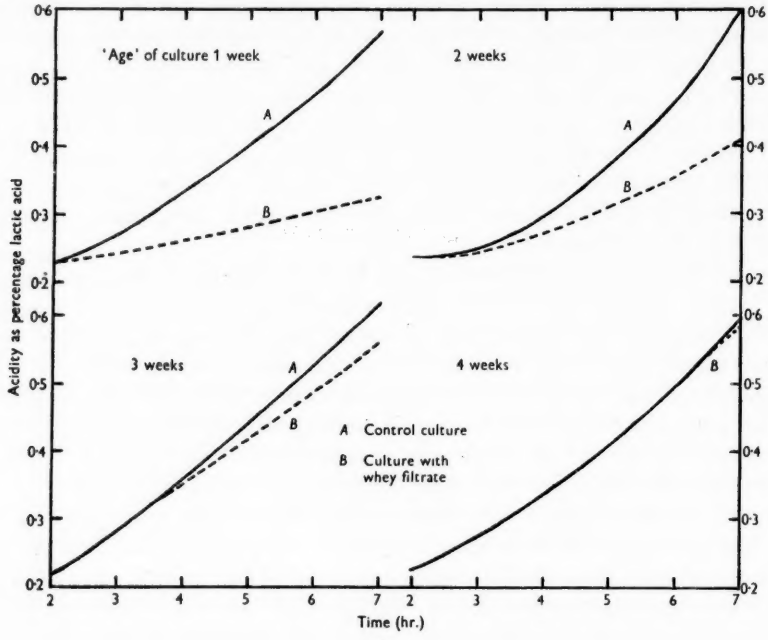


Fig. 1

the action of a variety of other phage races capable of attacking resistant and partially resistant HP cultures.

Examination of liquid cultures under the microscope showed a rather poor growth where the phage was taking effect on a 'young' culture as compared with a much heavier growth with an 'aged' culture, but the picture under the microscope with a 'young' culture did not always give a definite indication of partial lysis. Presumably the rather sensitive 'young' culture contained some organisms which were not adequately protected by phage 2, but this is pure speculation.

The occurrence of the new phage rather complicated the picture because in order to prevent its action on the starter in practice the use of an 'aged' phage-carrying culture was necessary, and the more 'aged' cultures were more liable to a sudden loss of the associated protective phage. The issue was finally resolved beyond doubt by the occurrence in one factory of another phage which had a clear-cut lytic action on both 'young' and 'aged' preparations, thus indicating quite clearly that HP/2+2 was not protected by phage 2 against all phage races capable of attacking resistant forms of HP.

DISCUSSION

The idea of 'internal' protection for a starter culture from the effects of contaminant phage is attractive because such a protected culture would not demand delicate handling, and consequently a less precise technique on the part of the factory manager or technician would be permissible. Unfortunately, the protection afforded to a culture by an associated phage is not wide enough for the purpose. In the present instance a culture was protected by the presence of an associated phage against attack by several different phage races, but ultimately a phage race capable of attacking it appeared in a commercial cheese factory. Even if a culture still more widely protected could be prepared, the multiplicity of phage races in nature suggests that eventually a phage capable of attacking the culture would gain access to it.

The phenomenon of associative growth of phages and streptococci probably plays a part from time to time in assisting the maintenance of activity in a mixed commercial starter. We have observed one instance in which a mixed starter behaving quite normally in a cheese factory regularly contained phage capable of attacking HP. One can easily imagine how a mixed starter, open to air-borne contamination with phage, may show a decrease in activity when a phage lyses one strain in the mixture. Thereafter resistant forms of the strain may develop with a consequent improvement in activity of the starter, and for a longer or shorter period the resistant strain may be protected against attack by other phages owing to the presence of the first phage. Thus the phenomenon may account for some of those periods of regular activity by a mixed starter maintained under crude conditions in commercial practice where it is subject to air-borne phage contamination. Where however a completely new mixed culture is prepared the initial period of freedom from trouble is more likely to be due to an absence in the surroundings of phage capable of attacking the streptococcal strains present in the mixture.

SUMMARY

Cultures of lactic streptococci partially resistant against a phage race and growing in regular association with that race are, as a result of the presence of the phage, protected from attack by some other races of phage. Such phage-carrying cultures were tried as cheese starters in commercial factories under conditions where no protection for the starter against air-borne phage was given. The results were quite satisfactory for several months, but eventually there appeared in the commercial factories races of phage which would attack the phage-carrying culture and thus depress or completely check acid development in the cheese vats. It seems likely that the large number of phage races which evidently exist in nature would make a complete protection along these lines very difficult to accomplish.

REFERENCES

(1) WHITEHEAD, H. R. & HUNTER, G. J. E. (1941). *J. Dairy Res.* **12**, 63.
(2) WHITEHEAD, H. R. & HUNTER, G. J. E. (1945). *J. Dairy Res.* **14**, 64.
(3) WHITEHEAD, H. R. & HUNTER, G. J. E. (1947). *J. Dairy Res.* **15**, 112.
(4) WHITEHEAD, H. R. & COX, G. A. (1936). *J. Dairy Res.* **7**, 55.
(5) HUNTER, G. J. E. (1947). *J. Hyg., Camb.*, **45**, 307.
(6) WHITEHEAD, H. R. & COX, G. A. (1932). *N. Z. J. Sci. Tech.* **13**, 304.
(7) HUNTER, G. J. E. (1946). *J. Hyg., Camb.*, **44**, 264.

(*MS. received for publication* 8 *March* 1949)

576.809.6

403. THE CHARACTERISTICS OF STREPTOCOCCAL PHAGES

INTERACTION BETWEEN DIFFERENT RACES

By G. J. E. HUNTER

Dairy Research Institute (N.Z.), Palmerston North, New Zealand

Throughout the work at this Institute on streptococcal phages, the only sure way found for isolating a fresh race of phage has been the exposure of a streptococcal culture to air-borne contamination. The use of a culture as a starter in a commercial cheese factory is the most convenient and effective way of ensuring daily exposure to air-borne phage because the milk, whey and curd in the cheese vat in which the starter is growing for several hours presents a large surface to the atmosphere. Even under these conditions, several months may elapse before a fresh phage race makes its appearance, but sooner or later a phage capable of attacking a given strain of *Streptococcus* is found in the cheese whey. Where several different phage races are capable of attacking a given 'single-strain' starter, the conditions determining which of the races shall establish itself are at present unknown. It is not simply a matter of one race being present in the surroundings and the others absent. In certain instances, where several phages are known to be present, one race was able to establish itself to the exclusion of the others, and, in fact, evidence has gradually emerged which seems to indicate that the presence of one race may interfere with the growth of another.

A particular *Str. cremoris* culture (HP) has been used very widely in commercial dairying in New Zealand. Whenever an isolation of phage is made from cheese whey or from cheese in which culture HP is the susceptible *Streptococcus*, phage race 2 is isolated (1). Yet it is known (1, 2) that several other races can all attack HP, some equally as strongly as race 2 when tested in the laboratory. When a resistant form of HP (prepared from the secondary growth obtained after lysis of culture HP by phage race 2) is exposed to infection by being used in a cheese vat, then these different phages readily appear. The modification of a culture by means of phage attack, and the creation of resistant forms, apparently permits other races to arise by virtue of the fact that the first phage is removed from the field of competition. Thus it is evident that other races are present in nature, but, where HP culture is concerned, they cannot compete with phage race 2. Repeated checking during the isolation of phages from whey, or cheese in which HP has been used as starter, has failed to show evidence of the development of polyvalent races such as 10 and 34 (1, 2). The specific phage race 2 invariably attacks culture HP in commercial practice.

This evidence from the field has been investigated only in one instance in the laboratory. Phage race 10 was isolated from cheese whey when a culture B (a completely resistant form of HP) was in use as starter in the vat. It has a wide range of lytic activity, and is able to lyse certain strains of lactic streptococci morphologically and biochemically different from the group to which HP belongs (1). When phage races 2 and 10, either in the form of plaques from an agar plate, or as undiluted phage preparations, are both added to a culture of HP in milk, the organisms are lysed after a period of growth, but no significant concentration of race 10 can be detected in the fluid medium. The large amount of detailed

work which is necessary to distinguish between phages in admixture, and to identify each phage with certainty, has so far prevented the collection of further evidence on this point.

A representative set of data obtained during laboratory trials to determine the extent of growth of phage races 2 and 10 in admixture on the susceptible culture HP is given in Table 1. The tests were made in tubes of sterilized skim milk containing the phages and inoculated with cultures HP or B. The action in milk was followed by microscopic examination of stained smears at intervals, and the amount of growth of the two phages in the lysed cultures was estimated by spotting dilutions of the cultures on agar plates previously spread with the appropriate organisms.

Table 1. *Growth of phages in admixture on susceptible organisms in milk*

Organism and phage race	Action in milk at 37° C.	Highest dilution of lysed culture showing plaques on	
		HP	B
HP + race 2	Lysis	10^{-10}	No plaques with neat preparation
HP + race 10	Lysis	10^{-5}	10^{-6}
HP + races 2 and 10	Lysis	10^{-10}	No plaques with neat preparation
B + race 10	Lysis	10^{-3}	10^{-6}

It is evident that although race 10 develops on culture HP, usually to give a lower final titre than race 2, it is unable to develop to any appreciable extent when competing against race 2. Further evidence that race 10 does not develop on HP in the presence of race 2 was obtained on examination of resistant organisms arising from lysed HP cultures after the action of mixtures of phages 2 and 10. The secondary cultures were resistant to race 2 but not to race 10.

Preliminary work has shown that all phage races capable of developing on culture HP are not so completely checked as race 10 by the presence of race 2. Some races develop equally with race 2, while others show varying degrees of retardation.

DISCUSSION

Growth interference amongst bacterial viruses is not a new phenomenon. In recent years, the effect has been described by Delbrück & Luria (3) and Delbrück (4), who showed that when different bacterial viruses grow on the same host, one cell liberates only the virus of one type. The results of the present work demonstrate that one member of a pair of streptococcal phage races interferes with the growth of another. The reason for the interference is not clear. Variation in optimum growth temperatures of different phages (5) might, under a given set of conditions (as in cheese manufacture), determine which phage race gained ascendancy, although on this basis it is difficult to see why the less favoured race should not grow at all. It seems more likely that the phenomenon is another aspect of that described by Hunter (2), in which a phage growing in association with a partially resistant strain of *Streptococcus* protects the culture from attack by other phage races. In both instances it seems possible that there is preferential adsorption of one phage race on the *Bacterium*, with consequent 'blocking' of the adsorption of the other races. In the phenomenon described in the present paper, lysis takes place because the organism is sensitive to the action of the adsorbed phage, whereas in the phenomenon of the phage-carrying culture lysis does not take place because the culture as a whole is partially resistant to the action of the adsorbed phage growing in association with it. The picture

is complicated by the fact that the 'adsorption' and 'blocking' effects in both cases are selective. Phage race 2, when adsorbed on the sensitive *Streptococcus*, is able to 'block' completely only certain races. Other races are partially interfered with, while others are not affected. Up to the present, therefore, no convincing evidence can be adduced in support of the adsorption hypothesis.

The phenomenon must be taken into account in dealing with crude phage-containing material, where in all probability several phage races are present. Unless several substrate organisms are used in the technique of phage isolation, one or more races may easily be overlooked.

SUMMARY

Although it is known that several phage races, capable of developing on a certain strain of *Streptococcus cremoris*, exist in the surroundings of commercial cheese factories in New Zealand, only one specific race is invariably isolated from cheese or cheese whey when the culture is used as a starter. Laboratory and field evidence suggests that this particular race has a special affinity for the organism, and that its presence interferes with the growth of other races which could develop in its absence.

REFERENCES

(1) Hunter, G. J. E. (1946). *J. Hyg., Camb.*, **44**, 264.
(2) Hunter, G. J. E. (1947). *J. Hyg., Camb.*, **45**, 307.
(3) Delbrück, M. & Luria, S. E. (1942). *Arch. Biochem.* **1**, 111.
(4) Delbrück, M. (1945). *J. Bact.* **50**, 151.
(5) Hunter, G. J. E. (1943). *J. Dairy Res.* **13**, 136.

(*MS. received for publication* 9 *May* 1949)

637.143.6

404. DETERMINATION OF THE SOLUBILITY OF ROLLER-DRIED MILK POWDER

By A. T. PARSONS

Government Laboratory, Clement's Inn Passage, Strand, London, W.C. 2

This note gives particulars of a method of determining the solubility of roller-dried milk powders which has been in use in the Department of the Government Chemist for some years. The figures obtained are empirical but are found to be a valuable aid in the comparative assessment of quality. The method is not claimed to be highly original, but appears to have aroused sufficient interest to justify publication. It is used in the Department for roller-dried milks only. For spray-dried powders a method is employed which measures the volume of deposit given on centrifuging similar to the Solubility Index test of the American Dry Milk Institute.

METHOD

(1) *Reconstitution*

Weigh out approximately 4 g. of powder into a 50 ml. boiling tube. Add 32 ml. of water at 50° C. Cork tube and shake for 10 sec. Place in a water-bath at 50° C. for 5 min. Shake for 1 min. In shaking make 4–6 double excursions of about 12 in. per sec. In dealing with separated powders use this liquid cooled to 20° C. for determining total solids I. In the case of full-cream and half-cream powders remove fat as described under (2).

(2) *Removal of cream (not applicable to separated powders)*

Completely fill a centrifuge tube with the hot reconstituted milk and centrifuge for 10 min. Cool in refrigerator until the fat is solid, but do not freeze the milk. Remove the fat layer with as little milk as possible by running a needle around the cake of fat and then removing it with a spoon-shaped spatula. Warm the milk to 20° C. Break up deposit with a rod or wire. Cork tube and shake well until the liquid appears homogeneous.

(3) *Total solids I*

Transfer about 2 ml. of the milk direct from (1) if separated, or after removal of the fat and redispersal of the precipitate in the case of full-cream and half-cream powder, to a tared covered metal dish and weigh. Let weight of liquid equal L_1. Place covered dish aside for drying with total solids II.

(4) *Total solids II*

Centrifuge tube again for 10 min.

Pipette about 2 ml. of upper layer into a second covered dish without disturbing sediment and weigh. Let weight of liquid be L_2.

(5) *Drying*

Uncover both dishes and place side by side on steam bath until apparently dry. Place in oven at 100° C. for 1½ hr. Cover dishes, cool and weigh.

Let weight of solids corresponding to L_1 and L_2 be S_1 and S_2. Then

$$\text{Solubility} = \frac{S_2/L_2}{S_1/L_1} \times 100\,\% = \frac{S_2 \times L_1 \times 100}{S_1 \times L_2}\,\%.$$

NOTES

The centrifuge tubes used have a total capacity of about 25 ml. The centrifuge is run at about 2000 r.p.m., and as the radius to the bottom of the rotating tube is about 17 cm. the precipitating force used is 769 times that due to gravity.

It is important that in the process of drying, the dishes containing total solids I and II should be treated in precisely the same manner.

The dishes used are of aluminium, about 6 cm. in diameter and 2·5 cm. deep. They are furnished with tight-fitting lids.

I wish to express my thanks to the Government Chemist for permission to publish this note.

(*MS. received for publication* 27 *April* 1949)

637.143.6

405. THE COMPOSITION OF THE SOLUBLE AND INSOLUBLE PORTIONS OF RECONSTITUTED MILK POWDERS

By R. WAITE and J. C. D. WHITE
Hannah Dairy Research Institute, Kirkhill, Ayr

INTRODUCTION

It is important that milk powder should be capable of being easily reconstituted in water without leaving an insoluble sediment. Many methods of measuring this property, usually termed the 'solubility' of the milk powder, have been proposed. In all of them the reconstituted powder is first centrifuged to sediment the insoluble material; thereafter the methods differ considerably, but most fall into one of two groups. In the first group are methods which depend on the measurement of the soluble total solids or protein in the dispersed phase, such as those of Supplee & Bellis (1), Hunziker (2), Wright (3), Howat, Smith, Waite & Wright (4) and Parsons (5) (see p. 388). The second group consists of methods which determine the weight or the volume of the insoluble, or more properly, non-dispersed sediment. Of this group, the methods of Marquardt (6), Lampitt & Hughes (7), Lampitt & Bushill (8), Cone & Ashworth (9) and that advocated by the American Dry Milk Institute (10) should be mentioned. Arguments can be propounded in favour of both types of determination, but it appeared to us that a better understanding of the way in which the major constituents of milk powders behaved when reconstituted was essential to a correct evaluation of the merits of the various methods.

To this end, six whole-milk powders, two manufactured by the roller process and four by different methods of spray-drying, were reconstituted under standard conditions and separation of the fat and sediment accelerated by centrifuging. (These samples have been taken to represent some of the different types of powder which are at present manufactured, but they are not necessarily typical of the particular type of plant on which they were prepared.) On centrifuging the reconstituted powder three layers are obtained, top or fat, middle or liquid and bottom or sediment. These layers were analysed for the major milk constituents. As one aim of good manufacture is to avoid any sediment, it was obviously important to examine this fraction in greater detail. To this end, five of the whole-milk and five separated-milk powders were used, and a sufficient bulk of sediment obtained by passing large volumes of the reconstituted milks through a Sharples super-centrifuge (14,500 × g). The sediments were washed, dried and analysed. The 'solubility' of each of the powders was measured by methods representative of the two groups and the validity of the results assessed.

EXPERIMENTAL

Each powder was first analysed for moisture, protein (total nitrogen × 6·38), fat and ash. The sum of these in percentage deducted from 100 was assumed to be lactose. There were no appreciable differences in the composition of the whole-milk powders nor of the separated powders and all were normal.

(*a*) *Analysis of fat and liquid layers.* The powder (4 g.) was weighed into a 50 ml. centrifuge tube of known weight and reconstituted with 36 ml. distilled water. In one

series of experiments the tube was held at 20° C. and in a second series at 50° C. for 5 min. It was then shaken for 1 min., cooled where necessary to 20° C. and centrifuged at 3000 r.p.m. ($1800 \times g$) for 15 min. This method of reconstitution, using 1 g. samples, has already been described in detail (4). After centrifuging, the tube was allowed to stand for 1 hr at 4° C. to harden the top layer which was then removed as completely as possible and weighed. The liquor was siphoned off and its weight found. Any residual fat on the walls of the tube was removed on a tared filter-paper and its weight added to that of the top layer. The tube was weighed again and the weight of sediment found. In order to provide sufficient material for analysis, eight tubes of the same powder were used in each determination and the fat and liquid layers bulked separately. Water, total nitrogen, fat and ash were determined on each of the bulked layers and lactose calculated by difference. The ash of each layer was analysed for calcium and phosphorus. The sediment in four of the eight tubes was treated in the manner described by Lampitt & Hughes (7) to provide data for calculation of powder solubility by their method. The composition of the sediments, in view of the very small amounts obtainable from spray-dried powders by this method of reconstitution, was calculated from the data of the fat and liquid layers and the analyses of the original powders. Sufficient sediment for analysis was obtained from the roller-dried powders, and the results agreed well with the data obtained by calculation.

(*b*) *Analysis of sediment.* 1 kg. of each powder, reconstituted at 20° C. in the same proportions and in a similar manner to the smaller samples, was run through the clarifier bowl of the Sharples super-centrifuge. This exposed the reconstituted milk to a field of $14{,}500 \times g$ for about 5 min. and gave a highly compressed sediment. The sediment was removed, mixed with 200 ml. distilled water at 20° C., shaken for about 1 min. and spun for 30 min. in the batch bowl of the Sharples. The mixing and washing was repeated twice again. The sediment was removed from the bowl and dehydrated by successive washings with 30, 50, 80 and 100% (v/v) ethanol, being spun for 15 min. with each concentration of alcohol. Finally, the sediment was dried *in vacuo* at room temperature over phosphorus pentoxide, allowed to reach moisture equilibrium by exposure to the laboratory air for 2 days and analysed as before for moisture, total nitrogen, fat and ash. Even with this treatment some small amount of occluded middle layer may have remained in the sediment, and any difference between the percentage sum of the constituents determined and 100 has been reported as lactose.

RESULTS

Table 1 gives the results of the work on the distribution of the major constituents among the three layers. The amount of each constituent appearing in each of the three layers is expressed as a percentage of the total amount in the reconstituted milk, and, since the figures for the sediments were obtained by difference, each horizontal line totals 100. Negative values shown for some of the sediment constituents at 50° C. give an indication of the experimental errors inherent in the technique employed. The partition of calcium and phosphorus between the layers is also given in Table 1, with figures in parentheses to indicate the percentage of each contributed by the liquid entrained in the fat and sediment layers, assuming such liquid to have the same composition as that of the middle layer. Thus the fat layer of the reconstituted roller-dried powder A contained 14·4% of the total calcium, but because 1·4% was contributed by the entrained liquid the true proportion of calcium in the fat layer was 13·0% of the total.

Table 2 shows the composition (as percentages) of the dry sediment produced in the

Table 1. *Percentage distribution of certain constituents of reconstituted whole-milk powders after centrifuging and the weights of the top, middle and sediment fractions*

Powder ...	Roller A			Roller B			Kestner			'Klim'			Krause			Milkal		
Layer ...	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment
Reconstitution at 20° C.																		
Water	4·1	77·2	18·7	1·0	79·9	19·1	1·9	91·9	6·2	1·0	94·7	4·3	1·3	96·7	2·0	1·3	96·5	2·2
Protein (N × 6·38)	16·8	23·1	60·1	2·5	63·8	33·7	6·5	61·6	31·9	3·3	89·4	7·3	3·5	92·5	4·0	3·1	92·5	4·4
Fat	68·7	5·3	26·0	83·2	11·0	5·8	20·3	56·8	22·9	30·7	67·2	2·1	40·0	57·2	2·8	45·1	54·2	0·7
Ash	9·1	59·4	31·5	1·8	73·2	25·0	3·6	73·6	22·8	2·0	92·5	5·5	1·9	97·1	1·0	1·9	92·5	5·6
Ca	14·4	25·8	59·8	2·4	61·7	35·9	6·5	70·7	22·8	2·2	85·7	12·1	2·2	93·5	4·3	3·1	94·9	2·0
	(1·4)*		(6·3)	(0·7)		(14·7)	(1·5)		(4·8)	(0·9)		(3·9)	(1·2)		(1·9)	(1·3)		(2·1)
P	13·2	34·2	52·6	3·7	62·6	33·7	6·6	81·6	11·8	2·7	83·8	13·5	2·6	97·4	0·0	2·6	94·9	2·5
	(1·8)		(8·2)	(0·8)		(15·0)	(1·7)		(5·5)	(0·9)		(3·8)	(1·3)		(2·0)	(1·3)		(2·2)
Wt. of each layer from 100 g. powder in 900 ml. water (g.)	63	737	200	33	775	192	26	906	68	19	940	41	24	956	20	25	954	21
Solubility (Howat *et al.*(4))		76			96			93			97			100			100	
Reconstitution at 50° C.																		
Water	0·8	83·6	15·6	0·7	97·9	1·4	0·6	98·3	1·1	0·9	98·0	1·1	1·3	97·9	0·8	0·9	98·4	0·7
Protein (N × 6·38)	2·1	50·4	47·5	0·9	92·9	6·2	1·6	82·7	15·6	2·9	98·4	−1·3	3·9	93·2	2·9	2·2	93·9	3·9
Fat	58·9	14·2	26·9	85·9	13·6	0·5	18·0	83·1	−1·1	54·9	37·9	7·2	48·1	51·1	0·8	52·3	46·3	1·4
Ash	1·4	72·2	26·4	0·8	98·1	1·1	1·1	99·4	−0·5	1·1	91·1	7·8	2·4	101·1	−3·5	1·3	98·6	0·1
Ca	1·7	49·1	49·2	0·7	96·4	2·9	1·3	99·0	−0·3	2·1	99·8	−1·9	3·4	96·5	0·1	1·8	98·0	0·2
	(0·5)		(9·2)	(0·7)		(1·1)	(0·5)		(1·1)	(1·0)		(1·1)	(1·3)		(0·8)	(0·9)		(0·7)
P	3·3	55·1	41·6	1·5	94·1	4·4	2·5	92·8	4·7	4·1	97·9	−2·0	5·6	95·2	−0·8	3·9	95·9	0·2
	(0·5)		(10·3)	(0·6)		(1·3)	(0·5)		(1·1)	(0·9)		(1·2)	(1·3)		(0·8)	(0·9)		(0·6)
Wt. of each layer from 100 g. powder in 900 ml. water (g.)	25	806	169	31	954	15	11	979	10	25	966	9	27	965	8	23	971	6
Solubility (Howat *et al.*(4))		91			98			100			100			100			100	

* Data in parentheses represent amounts of Ca and P contributed by permeating middle layer.

Negative values for some sediment constituents at 50° C. indicate the experimental errors inherent in the technique.

Table 2. *Analysis of sediments obtained by centrifuging reconstituted* (20° C.) *milk powders in the Sharples super-centrifuge* (g./100 g. *dry sediment*)

Powder ...	Roller		Kestner		'Klim'	Krause		Milkal		Gray-Jensen
	Whole A	Separated	Whole	Separated	Whole	Whole	Separated	Whole	Separated	Separated
Protein (N × 6·38)	76·0	79·7	86·8	86·6	83·9	86·3	87·0	83·7	85·4	84·5
Fat	10·7	1·8	2·4	0·3	5·8	1·2	0·3	2·4	0·2	0·7
Ash	9·3	8·6	9·1	9·3	8·9	9·8	10·2	9·2	9·6	10·1
Lactose (by diff.)	4·0	9·9	1·7	3·8	1·4	2·7	2·5	4·7	4·8	4·7
Ca	3·07	2·82	3·07	3·02	3·10	3·33	3·36	3·14	3·17	3·41
P	1·85	1·54	1·81	1·59	1·77	1·94	1·97	1·75	1·46	1·83
Ca/P ratio	1·66	1·83	1·70	1·90	1·75	1·72	1·71	1·79	2·17	1·86
Ca (g./100 g. ash)	33·0	32·6	33·7	32·3	34·8	34·0	33·0	34·1	33·1	33·7
P (g./100 g. ash)	19·9	17·4	19·9	17·2	19·9	19·8	19·4	19·0	15·4	17·8
Ash (g./100 g. protein)	12·2	10·8	10·5	10·7	10·6	11·4	11·7	11·0	11·2	11·9
Ca (g./100 g. protein)	4·08	3·51	3·57	3·47	3·70	3·83	3·91	3·70	3·75	4·03
P (g./100 g. protein)	2·50	1·88	2·07	1·85	2·15	2·20	2·30	2·15	1·76	2·13
Wt. of dry sediment obtained per 100 g. dry powder (g.)	6·8	16·0	0·9	1·2	1·4	0·7	0·9	1·2	1·0	3·0
Solubility of the powders (by method of Howat *et al.*(4))	76	81	93	100	97	100	100	100	99	98
Powder analysis:										
Ca (g./100 g. ash)	14·5	15·9	14·5	16·7	12·9	14·8	14·8	14·4	15·7	16·5
P (g./100 g. ash)	11·3	12·2	12·0	12·8	10·6	12·3	11·4	11·5	12·1	12·7
Ca/P ratio	1·28	1·31	1·21	1·26	1·23	1·20	1·26	1·26	1·28	1·33

Table 3. *Solubility of whole-milk powders as determined by various methods*

Powder ...	Roller A		Roller B		Kestner		'Klim'		Krause		Milkal	
Reconstituted at ...	20° C.	50° C.	20° C.	50° C.	20° C.	50° C.	20° C.	50° C.	20° C.	50° C.	20° C.	50° C.
	Solubility of the milk solids (%)											
Method:												
Howat *et al.*(4)	76	91	96	98	93	100	97	100	100	100	100	100
Lampitt & Hughes(7)	75	80	95	100	93	100	97	100	100	100	100	100
Parsons(5)	70	81	96	99	91	99	94	98	98	99	98	99
	Sediment volume (ml.)											
Amer. Dry Milk Institute(10)	10·0	(11·5)*	(4·0)	0·4	0·6	<0·1	0·3	<0·1	0·1	<0·1	0·1	<0·1
Howat *et al.*(4)	1·6	(2·4)	(3·0)	0·1	0·5	<0·1	<0·1	0·05	<0·1	<0·05	<0·1	<0·1.

* Parentheses indicate that the demarcation line between the middle layer and the sediment was indistinct.

Sharples super-centrifuge from five of the same whole-milk powders as reported in Table 1 and also from five separated-milk powders, four of which were manufactured in the same type of plant as four of the whole-milk powders. The calcium and phosphorus content of the ash of the original powders is included for comparison with that of the washed sediment ash.

Table 3 records the values for solubility of the six whole-milk powders as determined by methods representing the two groups previously mentioned; the volume of unwashed sediment obtained by the method of Howat *et al.*(4) is included for comparison.

DISCUSSION

General considerations. When whole-milk powder is reconstituted in water a number of physical changes take place concurrently. Lactose and the soluble salts go into true solution, fat is liberated from the particles, and the protein which has not been denatured

dissolves or becomes redispersed into a stable suspension. Where protein deterioration has taken place, as a result either of overheating during manufacture or bad storage conditions, the affected protein does not remain in suspension but slowly sediments. The behaviour of the fat when roller-dried powder is reconstituted in warm water differs markedly from that in milk made from spray-dried powder. The fat globules from roller powder are much larger than those from spray-dried powder, and they rise to the surface at a much greater rate, joining with others either on the way or at the surface to form an obvious layer of liquid fat. Any tendency which the fat in a reconstituted spray powder has to rise to the surface is more akin to the creaming of fresh milk than to fat separation *per se*. It would appear that the protein 'membrane' normally surrounding the fat globules in liquid milk is disrupted to a much greater extent in roller-drying than in spray-drying. King(11) has suggested that during roller-drying heat denaturation of the protein membrane surrounding each fat globule and also possibly mechanical fracture of the membrane by lactose crystals allow the globules to coalesce when the powder is reconstituted and so prevent the formation of a fine emulsion. In the spray-drying process there is little likelihood of the membrane being denatured by heat, and it is well known that in properly made and stored spray powder the lactose exists as an amorphous 'glass', whereas in roller-dried powders it is mainly crystalline.

If the reconstituted milk is centrifuged, the fat and non-dispersed material separate quickly and three fractions can be easily distinguished: the top, containing a high proportion of fat, the sediment, with a high proportion of protein, and a liquid middle layer. In practice, the middle layer permeates the top and bottom layers so that soluble constituents are present in both. Moreover, the 'insolubility' of the reconstituted powder, or, more properly, the amount of non-dispersible protein which may vary physically from an almost granular to a flocculent form, considerably affects the composition of the fat and sediment layers. If the powder is relatively 'insoluble' and contains a fair proportion of denatured protein, there is an increased chance that as the fat rises to the surface it will carry with it some insoluble protein. Conversely, fat globules may be trapped by the large bulk of insoluble protein as it sediments. If at the same time the fat globules are large and the degree of protein denaturation high, as in some roller powders, both types of entrainment will have a greater chance of occurring. In addition, some fat may never escape from particles of powder because of a covering of insoluble protein. These undissolved particles will probably sediment if they contain more protein than fat and rise to form part of the top layer if they contain more fat than protein. Again, since most milk powder is reconstituted by some form of shaking or mechanical agitation, the froth so produced may consist mainly of partially denatured protein (analogous to the process of 'whipping'). When such a liquid is centrifuged the froth collapses and some non-fatty material may remain on the surface layer. It is of interest to record that the reconstituted spray-dried powders frothed more than the roller-dried powders when shaken, and that after centrifuging the surface of the fat layers of the former was covered with a thin paper-like layer. In Fig. 1 an attempt has been made to depict the appearance in cross-section of the top layers of the reconstituted powders. It should be mentioned that the top layers of powders reconstituted at 20° C. often possessed a diffuse base of cream-like consistency, whereas at 50° C. the demarcation of the top layer from the liquid or middle layer was sharp. The spray-dried powders all showed evidence of a top skin of collapsed foam whereas the upper portion of the roller-dried top layers was a white, homogeneous, grainy plug. The Krause

and Milkal powders reconstituted at 20° C. appeared to have a top layer comprised of four parts, with a second protein-like skin interposed between two cream layers. To summarize, therefore, both the top fat layer and the sediment layer of centrifuged, reconstituted whole-milk powder may be expected to contain fat, lactose, soluble salts and protein, in amounts governed primarily by the manufacturing process and also by the methods employed in reconstituting and centrifuging.

The nature of the layers obtained on centrifuging the reconstituted powders. It will be seen from Table 1 that the six powders varied from a rather insoluble atmospheric roller-dried product through a very soluble roller powder, a low and an average solubility spray-dried

Powder	Temp. (°C.)	Thickness (mm.)	Appearance of top layer	Description
Roller A	20	13		White, homogeneous grain, no free fat
	50	2		White, homogeneous grain, slight trace of free fat
Roller B	20	2		Three layers: (*a*) white homogeneous grain; (*b*) clear, yellow, mobile fat; (*c*) loose white material
	50	4		Two layers: (*a*) white homogeneous grain; (*b*) mainly free fat containing some white solids
Kestner	20	6		Three layers: (*a*) top skin (collapsed foam); (*b*) white homogeneous grain; (*c*) creamy base
	50	2		Top skin over cream plug
'Klim'	20	2		Top skin over cream plug with fatty base
	50	2		Top skin over cream plug
Krause	20	3		Four layers: (*a* and *c*), protein-like skin; (*b* and *d*), yellowish creamy layer
	50	2		Top skin over cream plug
Milkal	20	3		Four layers: (*a* and *c*), protein-like skin; (*b* and *d*), yellowish creamy layer
	50	2		Top skin over cream plug

Fig. 1. Appearance of cross-section of the top layers after centrifuging reconstituted powder (4 g. in 36 ml. water) at 1800 × g.

powder to two highly soluble spray-dried powders. The considerable variation in the analytical figures for the three layers of these powders afford some indication of the changes occurring when whole-milk powder is reconstituted.

Comparing first the top layers of the powders reconstituted at 20° C., those from the roller powders weighed most and contained by far the most fat, although they differed considerably from each other. The protein content of the top layers was inversely related to the solubility of the powder, the highly soluble roller powder B containing less than the Kestner spray material. The amount of ash in each top layer was almost directly proportional to both the water and protein content of the layer.

The sediment layers grouped themselves somewhat similarly, those from the roller

powders being by far the greatest in weight and containing larger fractions of the total protein. Again the sediment of roller powder A, which had a relatively low solubility, differed considerably in composition from that of roller powder B. The weights of sediment and sediment protein from the spray powders varied inversely with their solubilities. The ash in these sediments appeared to depend more on the amount of protein in the sediment than on the amount of entrained liquor. The fat content of the sediments varied with the degree of solubility in much the same way as the protein content of the top layers.

The distribution of constituents in the middle layers was naturally dependent on that in the other two fractions. Where much protein was insoluble, as in roller powder A and to a lesser extent in roller powder B and the Kestner spray powder, the proportion of protein in the liquid was low; in the three more soluble spray powders the composition of the middle layers was very similar, the high proportion of all constituents indicating a stable suspension.

Before turning to the corresponding data at 50° C. the mechanism of reconstitution in cold water should first be considered in the light of the above results. Wright (3) suggested that milk-powder insolubility can be considered as the result of two types of protein change during the drying process, that due to wet heat leading to irreversible insolubility, and that due to dry heat which can be overcome by reconstituting at 50–60° C. Hence the insoluble protein in the top and sediment layers at 20° C. represents the sum of these two types of insolubility. The rather insoluble roller-dried powder A and to a lesser extent the Kestner spray powder afforded good examples of the mutual trapping of fat and insoluble protein, resulting in a high proportion of fat in the sediment and of protein in the surface layer. The very high proportion of fat in the top layer of roller-dried powder B shows that so long as the powder has a high solubility, i.e. little denatured protein, the quick rise of the large globules of non-emulsified fat prevents it being carried into the sediment. The well-emulsified state of the fat in the reconstituted Krause and Milkal powders and their high solubility resulted in a large proportion of the fat remaining in the middle liquid layer and very little protein appearing in the top layer.

If one now considers what is likely to be the result of reconstituting the powders at a higher temperature, it might be expected, if Wright's (3) theory is correct, that protein made insoluble by dry heat will be redispersed, giving less in both the top and bottom layers and that a smaller amount of sediment will entrap less fat. The corresponding increase of protein in the liquor might then have the power to stabilize more fat. If the amount of protein dispersed in the middle layer at the higher temperature was large, it might stabilize not only fat previously trapped in the larger sediment but also fat previously appearing in the top layer. The net result would then be a diminution of fat in the sediment and top layer and a corresponding increase in the liquid middle fraction.

Roller-dried powder A was more soluble at 50° C., but the amount of fat in the sediment remained unchanged despite the decrease in quantity of sediment protein. One explanation for this would be that the fat was associated with some of the irreversibly insoluble protein and was never actually liberated during reconstitution. If this were so, then the reversibly insoluble material would contribute little or nothing to the fat content of the sediment. This view is borne out by the behaviour of roller powder B, in which reconstitution at the higher temperature reduced the amount of protein in the sediment from 33·7 to 6·2% of the total, showing that most of the sediment protein at 20° C. was of the reversibly insoluble type. At 50° C., 0·5% of the total fat appeared in the sediment of this powder as against

5·8% at 20° C., indicating that at the lower temperature only 5·3% of the fat had been trapped by the flocculent, reversibly soluble protein. The same type of mechanism was apparently operative in the Kestner powder, although it is clear that the protein can become irreversibly insoluble without necessarily enclosing fat. In the two roller-dried powders and the Kestner spray-dried powder the smaller sedimentation of protein at 50° C. as compared with that at 20° C. enriched the liquid layer with that constituent and also stabilized more fat in this fraction. The reverse occurred in the three more soluble spray powders where a little more fat passed from the liquid layer to the top layer than at 20° C., possibly as the result of the mobility of the liquid fat and the decreased viscosity of the liquor.

To attribute all the difference in solubility at 50° C. to redispersion of 'reversibly' denatured protein may be to neglect the greater accessibility of the protein to water at the higher temperature. Indeed, recent work in this laboratory indicates that soaking a roller-dried whole-milk powder of low solubility in water at 20° C. for 16 hr. prior to reconstitution raised the solubility from 76 to 86%. The solubility of this powder at 50° C. was 91%. Thus raising the temperature of reconstitution appears to allow more intimate mixing of water with protein, partly perhaps because of fat liquefaction. This, in turn, results in redispersion of some protein which, although apparently non-dispersible at 20° C., might have been had the water reached it.

To summarize, therefore, it would appear that the greater the insolubility of a milk powder, the greater the likelihood of finding appreciable amounts of fat in the sediment and of protein in the top layer. As the temperature of reconstitution is raised, or the amount of soaking prior to reconstitution increased, the solids of the top layer will become more wholly fat and of the sediment more wholly protein, the amount of fat in the sediment at 50° C. being closely associated with the quantity of irreversibly insoluble protein.

The analysis of the washed sediments. The values given in Table 2 for the washed sediments obtained in a high gravitational field ($14{,}500 \times g$) from whole- and separated-milk powders are very similar, particularly when allowance is made for the fat content of the former. Again, the relatively low solubility of the roller powders resulted in the inclusion of considerable amounts of fat even after three washings with water. When a powder has a high solubility, as in the spray-dried material, the washed sediment (whose volume is used as an index of solubility in the American Dry Milk Institute's method) will consist very largely of protein, although it has previously been shown (4) that the amount of sediment will decrease with the amount of washing to which it is subjected, rapidly at first and more slowly after the third washing.

The distribution of calcium and phosphorus. Although not of direct importance in considering solubility, the distribution of calcium and phosphorus between the various fractions is of theoretical interest. It can be seen in Table 1 that the true calcium and phosphorus content of the top and sediment layers was roughly proportional to the amount of protein in each, at both 20 and 50° C. In the washed sediments after centrifuging in the Sharples machine there was great similarity between the ratio of calcium to phosphorus (Table 2) from powders of different manufacture, the average ratios for the sediments of the whole-milk powders being 1·72 and of the separated-milk powders 1·89. In the original milk powders these average ratios were much less, 1·24 and 1·30 respectively, indicating roughly a 40% increase of calcium relative to phosphorus in the washed sediments. Moreover, in the sediments the proportion of both calcium and phosphorus to other constituents was considerably greater than in the original powders. This is best seen by

expressing calcium and phosphorus as a percentage of the ash when it is evident that the calcium content of the sediment ash was approximately double that of the ash of the original powder and that the phosphorus content was about 50% greater. In addition, the amounts of ash, calcium and phosphorus per 100 g. *protein* in the dry sediments were remarkably similar for all powders (Table 2). The explanation would appear to be that these washed sediments consisted mainly of denatured protein, and that the calcium and phosphorus were mainly that bound to the calcium caseinate and forming part of it. Ling's investigations (12, 13) lead to the conclusion that one-third of the calcium and phosphorus in milk is present in the form of soluble mono-calcium hydrogen phosphate. Of the remainder, 20% of the original calcium and 19% of the original phosphorus form part of the calcium caseinate micelles which have associated with them colloidal tricalcium phosphate. This tricalcium phosphate accounts for 47% of the calcium and 30% of the phosphorus, the residual phosphorus occurring mainly in the ester form. Other workers, Pyne (14) and Eilers, Saal & van der Waarden (15), are also of the opinion that the earlier concept of all the phosphate being present as mono-calcium hydrogen phosphate was wrong, and that the colloidal phosphate probably exists as tricalcium phosphate bound in some way to the calcium caseinate. The analysis by Ramsdell & Whittier (16) of the sediment obtained by subjecting fresh liquid milk to a very high centrifugal force supports the view that a calcium caseinate-tricalcium phosphate complex (consisting of 4·8% of tricalcium phosphate and 95·2% calcium caseinate) is present in milk. In such a complex, the ratio of calcium to phosphorus is 1·76, which is similar to the values of 1·72 and 1·89 obtained for the washed sediments in the present work. Thus the work described here supports the view that the colloidal phosphate associated with calcium caseinate is the tricalcium compound.

Methods of measuring solubility. Of the various methods of measuring solubility already mentioned, most give a reasonably accurate result with powders of high solubility such as a good spray-dried powder, but when applied to roller powders or to spray-dried powders of low solubility none of the methods give accurate results. The results in Table 3 may be critically considered in light of the knowledge of the distribution of constituents between the three layers.

The method of Howat *et al.* (4) includes the top layer in the soluble portion, whereas, in fact, up to 8% of the layer may consist of insoluble protein and results may be too high. In this method the top layer is removed, the liquid decanted and total solids measured in the mixed top and liquid layers, but as can be seen, the sediment often contains a considerable quantity of liquid, the diluting effect of which will be lost. Thus when the sediment is large the solubility calculated from the total solids in the top and liquid layers will be erroneously high. This will be counteracted to some extent by the fact that the sediment contains some fat which is not taken into consideration, but it should be remembered that at 50° C. (for roller powders at least) the fat appears to be closely associated with the irreversibly insoluble protein, and is, to all intents and purposes, itself 'insoluble'. The methods of Lampitt & Hughes (7) and Lampitt & Bushill (8) measure the weight of solids in the sediment layer. In both methods allowance is made for the effect of the permeating middle layer and in the second method for entrapped fat also, but insoluble protein will be lost in the discarded top layer resulting in too high a solubility value. The fact that in the method of Lampitt & Hughes no allowance is made for fat entrapped in the sediment will help to counterbalance the loss of insoluble protein in the top layer.

Results obtained by the method of Cone & Ashworth (9), in which also the weight of insoluble material is measured, will be too low, since the fat in both the sediment and top layers is calculated as insoluble material. Moreover, Cone & Ashworth assume that a single washing is sufficient to free the sediment of material from the permeating middle layer, whereas it has been shown (4) that at least three and possibly more washings are necessary. In Parsons's method (5), the top layer is discarded and the solubility expressed as the percentage ratio of solids in the middle layer to the total solids of the middle plus sediment layers, the errors compensating each other to some extent. In discarding the top layer the percentage of total solids in the combined middle and sediment layers will be low, but when the insoluble material is re-sedimented, the solids content of the supernatant liquid will also be low. Unless the sediment contains much fat when the top layer contains little protein or vice versa, results by this method should be near the true solubility of the powder.

The inadequacy of the sediment volume method of measuring milk-powder solubility is well demonstrated in roller powder A, where the sediment volume at 20° C. by both the American Dry Milk Institute's (10) method and that recorded during the solubility determination by the method of Howat *et al.* (4) was less than at 50° C. The sediment at 20° C. was dense and compact, whereas the sediment at 50° C. was loose and more aqueous; moreover, the top layer at 20° C. contained an appreciable amount of insoluble protein which, had it not been carried up by the fat, would probably have appeared in the sediment. It has often been noticed that sediments from different reconstituted powders may differ in density and mode of packing even when other experimental conditions are kept constant. It would appear that the sediment volume method is best suited for powders in which the amount of sediment is normally small, as in most spray-dried powders. Under these circumstances it can afford a sensitive index of manufacturing conditions. The sources of errors which have been mentioned and which can be seen to be common to the methods considered are minimized when the powder is reconstituted at 50–60° C., and unless a measure of the reversibly insoluble protein is required, solubility determinations at 20° C. even with soaking prior to reconstitution should be avoided, particularly for roller-dried powders.

If a fairly rapid method is required which is equally applicable to both whole and separated, roller- and spray-dried powders, that of Parsons (5) would appear to be the most suitable.

SUMMARY

1. The mode of distribution of the constituents of centrifuged reconstituted whole-milk powder manufactured by different processes has been determined at 20 and 50° C.

2. Where considerable 'insolubility' exists, protein is carried into the fat layer and fat into the sediment layer in quantities directly related to the degree of 'insolubility'. The fat in the sediment of roller powders appears to be associated almost entirely with the protein which remains insoluble at 50° C.

3. The sediments from reconstituted whole- and separated-milk powders made by different manufacturing processes have been obtained in bulk by vigorous centrifuging ($14{,}500 \times g$). Analysis of these sediments reveals considerable similarity between them, and it is concluded that a well-washed sediment would consist mainly of denatured calcium caseinate together with calcium and phosphorus in the same proportions as in normal tricalcium phosphate, $Ca_3(PO_4)_2$, the calcium caseinate and most of the calcium phosphate probably being in the form of a casein-phosphate complex.

4. The accuracy of some published methods of measuring milk powder solubility has been considered, and the inaccuracies arising from the non-homogeneity of the fat and sediment layers are stressed.

The authors wish to thank Mr F. C. White, of the Ministry of Food, and Mr W. B. Barbour, of the Scottish Milk Powder Co. Ltd., for the supply of samples of milk powder.

REFERENCES

(1) SUPPLEE, G. C. & BELLIS, B. (1925). *J. Dairy Sci.* **8**, 39.
(2) HUNZIKER, O. F. (1926). *Condensed Milk and Milk Powder*, 4th ed. La Grange, Illinois: the author.
(3) WRIGHT, N. C. (1932). *J. Dairy Res.* **4**, 122.
(4) HOWAT, G. R., SMITH, J. A. B., WAITE, R. & WRIGHT, N. C. (1939). *J. Dairy Res.* **10**, 498.
(5) PARSONS, A. T. (1949). *J. Dairy Res.* **16**, 377.
(6) MARQUARDT, J. C. (1920). *Ice Cr. Tr. J.* **16**, 4.
(7) LAMPITT, L. H. & HUGHES, E. B. (1924). *Analyst*, **49**, 176.
(8) LAMPITT, L. H. & BUSHILL, J. H. (1931). *Analyst*, **56**, 781.
(9) CONE, J. F. & ASHWORTH, U. S. (1947). *J. Dairy Sci.* **30**, 463.
(10) AMERICAN DRY MILK INSTITUTE INC. (1948). *Bull. Amer. Dry Milk Inst.* p. 911.
(11) KING, N. (1948). *Netherlands Milk and Dairy J.* **2**, 137.
(12) LING, E. R. (1936). *J. Dairy Res.* **7**, 145.
(13) LING, E. R. (1937). *J. Dairy Res.* **8**, 173.
(14) PYNE, G. I. (1934). *Biochem. J.* **28**, 940.
(15) EILERS, H., SAAL, R. N. J. & VAN DER WAARDEN, M. (1947). *Research in Holland. Chemical and Physical Investigations on Dairy Products.* Amsterdam: Elsevier.
(16) RAMSDELL, G. A. & WHITTIER, E. O. (1944). *J. biol. Chem.* **154**, 413.

(*MS. received for publication* 25 *June* 1949)

637.0:54+53

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION C. DAIRY CHEMISTRY AND PHYSICS

PART I. CHEMISTRY AND PHYSICAL CHEMISTRY
Introduction
I. Composition of milk and milk products:
(a) Milk
(b) Butter
(c) Cheese
(d) Dried milk
II. Chemistry of milk constituents:
(a) Fat
(b) Nitrogenous compounds
(c) Lactose
(d) Minerals
(e) Miscellaneous
(f) Enzymes
III. Physical chemistry
IV. Processing and manufacture
V. Defects of milk and milk products:
(a) Adulteration and its detection
(b) Flavour defects
VI. Analysis of milk and milk products:
(a) Milk
(b) Butter
(c) Cheese
(d) Dried milk
References:
(a) Arranged numerically
(b) Arranged alphabetically.
PART II. RHEOLOGY

PART I. CHEMISTRY AND PHYSICAL CHEMISTRY

INTRODUCTION

Seven years have elapsed since the last review of the progress of dairy chemistry (1) was published, and the volume of literature to be considered for the present review was so large that it was necessary to exercise a certain measure of selection and to give much less space to each paper than was possible in the earlier biennial reviews. The present review covers the period 1942–7, and also includes some earlier papers which, owing to wartime difficulties, were not available for the last review. The authors apologize for any omissions and for giving to papers of merit undeservedly brief mention. In preparing this review, *Dairy Science Abstracts* has been consulted freely, and the authors are greatly indebted to Mr W. G. Sutton, Director of the Commonwealth Bureau of Dairy Science, for the facilities placed at their disposal. They also wish to express their sincerest thanks to Dr S. J. Rowland for much valuable help and advice.

I. COMPOSITION OF MILK AND MILK PRODUCTS

(a) *Milk*

Cows' milk

The composition and properties of colostrum have been reviewed in detail by Houdinière (2). Mukherjee, Swaminathan & Viswanath (3) have observed the characteristic variations of the major milk constituents associated with the transition from pre-milk to colostrum and from colostrum to normal milk, with animals of the Sahiiwal breed.

Further attention has been given to the causes of variation in the solids-not-fat of milk. Riddet, Campbell, McDowall & Cox (4) have presented a valuable study of the relation of the plane of nutrition of the cow to milk production and milk composition in New Zealand. By two series of trials on the double reversal system, they have shown that changing cows from normal full rations to rations providing normal maintenance and only one-half normal production requirements caused a definite fall, ranging from 0·3 to 0·5 in the percentage of solids-not-fat in the milk. The fall affected mainly the protein content of the milk. There was no consistent effect on the fat percentage, but the iodine and saponification values of the fat rose.

Rowland (5) has described the increasing occurrence of milk with a low content of solids-not-fat during the winter months of the war years. Data obtained from one dairying area in 1942 and 1943 are presented to illustrate the high incidence of genuine herd milk containing less than the

presumptive legal standard of 8·5% solids-not-fat in the months of January to April. The solids-not-fat content improved rapidly to normal levels when the herds received ample young grass in April or May. The low content during the later winter months was ascribed to a low plane of nutrition of the herds at that period under the wartime conditions of a much reduced supply of concentrates and the need for increased reliance on home-grown feeding stuffs. In a later paper, Rowland (6) has discussed further the problem of low solids-not-fat, dealing with the general decline in solids-not-fat which had occurred over about the last 20 years, and stressing the particular significance of genetic and nutritional factors among the various factors influencing the percentage of solids-not-fat. This paper also gives an outline of an experiment to ascertain the effect of known separate deficiencies of energy and of protein in the feed of the cow on the composition of milk. Rations providing, for both maintenance and production, (*a*) starch equivalent and protein equivalent according to normal standards, (*b*) normal starch equivalent, but only 60% of normal protein equivalent, and (*c*) normal protein equivalent but only 75% of normal starch equivalent, were fed to each of eighteen shorthorn cows; each ration was fed for 5 weeks according to a latin square design. Both the low-energy and low-protein rations appreciably reduced the solids-not-fat content of the milk, the low-energy ration to the greater extent. The following average results were obtained for the percentages of solids-not-fat, protein and lactose respectively: normal ration, 8·68, 3·07 and 4·71; low-protein ration, 8·50, 2·95 and 4·65; low-energy ration, 8·34, 2·86 and 4·61, the differences being statistically significant. Two-thirds of the fall in solids-not-fat was due to milk protein and one-third to lactose.

The low solids-not-fat content of genuine milk has also been noted in South Africa by Davel (7), Bakalor (8) and van Rensburg (9), in East Africa by Purchase & Reverberi (10) and in the United States of America by Herman & Peterson (11). The low values have been related variously to breed (8, 10), season (or temperature) (9, 11) and nutrition (7, 8).

General analytical studies of milk composition have been made in Brazil by Amaral Rogick (12), in Norway by Mork (13), and in Switzerland by Kästli (14) and Kästli & Binz (15). In a survey of the fat content of Swiss milk, before and during the war, Mosimann (16, 17) found it to have been unaffected by the wartime shortage of feeding stuffs. The variability in the fat content of milk from individual cows is emphasized. Kästli (18) has discussed individual variations in fat content in connexion with the difficulty this introduces in proving cases of fat abstraction.

Thomé (19) has recorded a case of drastic reduction in the milk-fat percentage on turning a herd of cows out to spring pasture. The average fat level for fourteen cows fell from 3·2 to 1·4%, with individual values as low as 0·4%. Normal fat levels were regained after 2 weeks. In other respects the milks were normal. McClymont & Paxton (20) noted a reduction from more than 4 to less than 3% in the milk-fat content of a group of six cows grazed on young oats supplemented only with concentrates. When the animals were provided with adequate roughage, the fat content returned to its normal level. A control group of cows was provided with ample roughage throughout and maintained a normal fat level. Leroy & Bonnet (21) obtained a fall in the fat content of milk of 11%, and in the fat yield of 23%, by a reduction in the dietary fat intake by replacement of linseed and rapeseed in the ration with the corresponding cakes. Filipovič (22) has determined the water content of the milk of cows when stall-fed and during and after transition to pasture. A further statistical treatment of his data is given by the author (23) and by Mikič (24). The effect of feeding cottonseed and cottonseed meal respectively on milk composition has been investigated by Davis & Harland (25) and Miller & Wise (26).

Heinemann (27) has shown an inverse relationship to exist between air temperature and the fat and solids-not-fat content of milk. Yska (28) observed that the difference between the fat content of the morning and evening milk of individual cows was increased during a heat wave. Koestler, Kägi, Lehmann & Wegmüller (29) found the movement of herds from valley to high alpine pasture to have no marked effect on milk composition other than to cause a decrease in the volatile acid and an increase in the unsaturated acid content of the fat.

Seasonal variations in the solids-not-fat and fat content of market milk have been studied by Rees (30), while Overman (31) has discussed monthly variations using his earlier data [*Bull. Ill. agric. Exp. Sta.* nos. 325 (1929) and 457 (1939)].

Moore & Morrow (32) have reported that Jersey milk is richer in all major constituents than Holstein milk, with a considerable overlap between the breeds in the proteose-peptone and non-protein nitrogen fractions.

A statistical analysis of data collected by Bonnier, Hansson & Jarl (33) did not confirm the linear relationship between calorie content of milk and percentage of fat obtained earlier by Gaines & Overman [*J. Dairy Sci.* **21**, 261 (1938)]. Neseni & Körprich (34) have studied the lactational trends of the total nitrogen, casein, albumin and globulin content of milk and have determined the correlation between the percentages of these constituents and the fat content of milk.

Mean values of 0·47, 1·29 and 4·45 volumes % for the oxygen, nitrogen and carbon dioxide content respectively of sixty-three samples of raw milk have been reported by Noll & Supplee (35).

Milk of other species

Ghosh & Datta Roy (36) have studied the composition of buffalo milk in relation to the detection of adulterated samples passed as cows' milk. Anantakrishnan, Dastur & Kothavalla (37) have compared the composition of samples of mixed cows' and mixed buffaloes' milk. Figures for the composition of the milk of twenty-four goats, from four breeds and of varying age and stage of lactation, have been provided by Holmes, Kuzmeski, Lindquist & Rodman (38). A detailed analytical study of the composition of sows' colostrum and milk has been made by Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (39). The results obtained are probably the most reliable so far available, the volume of milk drawn per milking being considerably greater than hitherto as the result of stimulation of 'let-down' of milk by oxytocin. Holmes, Spelman, Smith & Kuzmeski (40) have compared the composition of the milks from one Palomino and four Percheron mares.

(*b*) *Butter*

In an attempt to account for the different consistencies of butters from different areas of Holland, Adriani, Tamsma, Vogel & Groot (41) have made an extensive study of factors influencing the refractive index of butterfat, using this as a guide to the firmness.

Sandelin (42) obtained an average air content for 1100 samples of butter of 2·76 ml./100 g. butter. Large butter grains were found to favour the inclusion of air during churning. Thiel (43) has reported values of 0·19 % at 40° C., 0·26 % at 60° C., 0·36 % at 80° C., and 0·47 % at 95° C. for the solubility of water in butterfat. Addition of phosphatides to the fat or addition of salt, dilute acid or alkali to the aqueous phase produced no change in solubility, but the solubility of water was slightly increased when 2 % of mixed fatty acids were dissolved in the butterfat. Sörensen (44) determined the copper content of eighty-five samples of Danish butter received for grading, and estimated that 2–3 % of all samples sent in annually for grading contained more than 0·2 p.p.m. High copper values were most frequent during the grazing season. Storgårds (45) has noted an increase in the iron and copper content of Finnish butter as a result of the war. He reports average values for 140 samples as 2·32 p.p.m. for iron and 0·81 p.p.m. for copper.

Caserio (46) found a Polenske value $>2{\cdot}0$, a 'refractive index' at 35° C. <46, and a Reichert-Meissl value <26 to be characteristic for whey butter and to form a suitable basis for distinguishing it from creamery butter.

(*c*) *Cheese*

Analytical data for several types of Roumanian cheese have been provided by Angelescu (47). Taha El-Katib (48) has shown that Egyptian cheese made from hand-skimmed milk varies widely in its chemical composition. Only small variations in the fat and salt content of different Tilsit cheese of the same batch have been noted by Schwarz & Mumm (49). They observed a tendency for the fat content of the dry matter to rise as ripening progressed, but consider that this may be due to a loss of volatile ripening products during the dry-matter determination. Koestler (50) found an open structure, a high water and fat content, a high rate of moisture evaporation and a high storage temperature to contribute to high fat leakage in cheese. More fat was expressed from the outer than from the inner portions. Old cheese tended to express the least fat. Zolli-

kofer(51) has given details of the nitrogen distribution in samples of more than forty varieties of cheese. Csiszár, Bakos & Tomka(52) obtained values ranging from 0·10 to 8·00 (average 3·25) mg./100 g. for the acetylmethylcarbinol content and values ranging from 0·00 to 1·00 (average 0·15) mg./100 g. for the diacetyl content of ninety-two samples of cheese of eight different varieties. No relationship was observed between the quality of the cheese and its diacetyl and acetylmethylcarbinol content.

Sproule(53) has determined the distribution of the calcium and phosphorus present in milk, in the first whey, press whey, and pressed cheese during the manufacture of Cheddar cheese. Average figures for calcium were 41·98, 0·98 and 60·46%, and for phosphorus 47·58, 0·72 and 52·46% respectively. Irvine, Bryant, Sproule, Jackson, Crook & Johnstone(54) have made an extensive study of the retention of calcium and phosphorus in Cheddar cheese. Cheese made at monthly intervals for a year from milk obtained from a mixed herd of cows contained, on the average, 0·774% of calcium, or 61·3% of the milk calcium, and 0·465% phosphorus, or 53·1% of the milk phosphorus. Monthly variations in calcium and phosphorus were only slight. Cheese made from the milk of cows grazing in areas with different types of soil formation had similar calcium and phosphorus contents. Pasteurization of milk caused increase in retention of phosphorus by the cheese(55). Values for the calcium and phosphorus content of cream, cottage, brick and blue cheese(56), cottage cheese(57), and Cheddar, Swiss, Edam, blue-veined, cottage and cream cheese(58) have been reported. Zahrndt, Lane & Hammer(58) have observed that the differences in calcium and phosphorus content of different varieties of cheese agree in general with the differences in manufacturing procedure and particularly with the degree of acid development. Schutz(59) has proposed a classification of cheese based on the calcium content of the dry, fat-free and ash-free material. Cheese with 2·5% calcium or more is classified as rennet cheese; cheese with 0·5% calcium or less as soured milk cheese, and cheese with intermediate calcium contents as rennet-acid cheese, the rennet action being assumed to decrease by 5% for every 0·1% decrease in calcium content below 2·5%.

Hoecker & Hammer(60) have investigated changes in the moisture, pH and salt content of the surface and inner portions of Cheddar cheese curd at various stages of manufacture up to 24 hr. after its removal from the press. At hooping, about 1 hr. after salting, salt content of the curd was high near the surface and low in the centre. Salt migration was rapid during the first 15 hr. of pressing. There was little difference between the salt content near the surface and that at the centre 24 hr. after the cheese had been removed from the press. In comparative experiments, Sirks(61) has observed that protein breakdown in Edam cheese was most rapid with cheese of high pH.

(*d*) *Dried milk*

Ashworth & Van Orden(62) have determined the total, casein, albumin plus globulin, proteose-peptone, non-protein and ammonia-nitrogen fractions of thirty-two samples of spray-dried skim-milk powder. Most of the milk had been pre-heated before drying, and when allowance is made for the resulting denaturation of albumin and globulin and the co-precipitation of the denatured proteins with casein, the values obtained agree well with those for fresh skim milk. Only four of the samples contained a measurable amount of un-denatured albumin and globulin, the maximum value observed being only 4·1% of the total nitrogen. Heinemann(63) obtained an average value of 2·00% (range 1·88–2·09) for the citric acid content of twenty-seven samples of dried skim milk collected throughout 1943. The citric acid content, determined by oxidation with perchloratocerate, was highest in March and April and lowest in November. A further eleven samples, all taken in July from different sources, showed a range of from 1·83 to 2·08% (average 1·96).

The titratable acidity and lactic acid content of samples of commercial dried skim milk obtained from Michigan processing plants immediately after manufacture have been determined by Gould & Skiver(64). The mean lactic acid contents, expressed in terms of reconstituted milk, were: dried milk, 0·018% (0·005–0·039) in summer and 0·008% (0·003–0·025) in winter; evaporated milk, 0·013% (0·008–0·022) in summer and 0·006% (0·004–0·013) in winter. On the basis of these data, a limiting standard of 0·015% of lactic acid is proposed for grading. Acharya & Devadatta(65)

have investigated the partition of phosphorus in two commercial samples of whole buffalo-milk powder.

The 'creaming' of the fat in *dahi* prepared from boiled cows' or buffaloes' milk, pasteurized or raw milk, has been studied by Anantakrishnan & Kothavalla (66).

II. Chemistry of milk constituents

(*a*) *Fat*

Anantakrishnan, Bhale & Paul (67) have reported that colostral fat differs appreciably from milk fat in containing smaller quantities of the various volatile fatty acids and more oleic acid. Up to the tenth day of lactation, an increase in the C_4, C_6 and C_8 acids at the expense of oleic acid was noted. However, the colostral fat of a single cow examined by Baldwin & Longenecker (68) did not differ essentially in fatty acid composition from values reported for typical butterfat.

Hilditch & Jasperson (69) have established the presence of octadecadienoic acids in cows' and goats' butterfat. The vaccenic acid content of butterfat and variations in its amount have been studied by Brouwer & Jonker-Scheffener (70). They record values ranging from 4·93% with cows on spring pasture to 1·28% on a winter ration. Geyer, Nath, Barki, Elvehjem & Hart (71), using a different method, report that butterfat contains from 0·5 to 0·7% of vaccenic acid. Mulder (72) has observed that the fat present in strippings and in cream has lower refractometer and iodine values and a higher melting-point than fat isolated from the corresponding fore-milk and skim-milk. Jack & Henderson (73) have fractionated butterfat by successive precipitation from pentane at low temperatures, and have analysed the fractions. In a later paper (74), they present evidence in support of Hilditch's suggestion that the component fatty acids are not distributed at random, but that each fatty acid is distributed as widely as possible among all the glyceride molecules.

Preliminary studies by Gould (75) suggest that only the long-chain acids are involved in the lipolysis of milk fat.

Hilditch & Jasperson (76) have made an interesting study of the influence of dietary fat of varying unsaturation on the component acids of butterfat. The ingestion of groundnut oil (iodine value 88) and of hydrogenated groundnut oil (iodine value 45), as an addition to a basal ration, increased the amount of oleo-glycerides in the milk fat and decreased the butyric-decanoic glycerides, the hydrogenated oil also increasing the stearo-glycerides. The ingestion of palm-kernel oil (iodine value 17) increased the lauro- and myristic-glycerides and decreased the palmito- and oleo-glycerides. Ingestion of hydrogenated groundnut oil (iodine value 17) did not appreciably alter the composition. Leroy & Bonnet (21) have observed that the fat present in grass appears to be specially suitable in chemical composition for transformation by the cow into butterfat. A detailed study of the effect of feed, with particular reference to pasture, on the iodine number of butterfat has been made by Sjollema (77). It is shown that the iodine number increased directly with the crude protein content of the grass.

Fat constants for Danish, Australian and Swiss butter have been reported (78, 79, 80). Variation of these values with feed and season are recorded in the above papers and in (29). Schloemer (81) has dealt specifically with the seasonal variations of the butyric acid number of butterfat, which were found to be small. Rauch & Schloemer (82) obtained a low negative correlation between refractive index and butyric acid number. Correlation coefficients and regression lines for a number of physical and chemical constants of butterfat have been provided by Van der Burgh, Brouwer & Koppejan (83).

The presence of squalene in butterfat to the extent of 7 mg./100 g. of fat has been reported by Fitelson (84). Hoecker & Hammer (85) have studied the distribution of diacetyl- and acetyl methyl-carbinol between the fatty and aqueous phase of butter. Hedrick & Hammer (86) and Hedrick (87) have shown that, using a standard starter culture, production of flavour during cream ripening was enhanced by the addition of citric acid, by high acidity and by agitation. They did not observe any relationship between the production of diacetyl and that of acetylmethylcarbinol, nor was their final concentration in butter related to their concentration in cream. Hedrick (87)

has also studied the development of diacetyl- and acetylmethylcarbinol in unsalted butters stored under various conditions.

Hilditch & Meara (88), Baldwin & Longenecker (89) and Brown & Orians (90) have studied the fatty acid composition of human milk fat. The proportions of saturated and unsaturated fatty acids were nearly equal, whereas in cow's milk fat the saturated acids predominate. The saturated acids were composed principally of palmitic acid, with smaller amounts of stearic and myristic and the lower acids lauric and decanoic. In contrast to butterfat, butyric and other acids below decanoic were absent. The unsaturated acids consisted principally of oleic acid, with smaller quantities of hexa-, tetra- and octadecadienoic acids and unsaturated acids of the C_{20} and C_{22} series. Hilditch & Meara (91) found that of the glycerides of human milk fat, 9·1% were fully saturated, 39·6% had one, 42·7% two and 8·6% three unsaturated fatty acid radicles. Baldwin & Longenecker (89) have observed that, in the human, the colostral fat is richer in phospholipids than that of later milk.

A progressive drop in the oleic acid content and rise in the butyric acid content of the fat secreted by the buffalo during the first 10 days of lactation has been observed by Anantakrishnan, Bhale, Paul & Rangaswamy (92). The fatty acid and glyceride composition of Indian buffalo milk fat has been studied by Achaya & Banerjee (93, 94). The same authors (95) have shown that Indian (tropical) animal fats have, in general, a higher content of saturated fatty acids (mainly palmitic acid) than the European fats analysed by Hilditch.

Fat constants and fatty acid composition for one sample each of goats', ewes' and mares' milk (96) and the component fatty acids of sows' milk (97) have been recorded. Zeisset & Grossfeld (98) have provided correlation coefficients for various constants of the fat of goats' milk, based on analytical data for 169 samples.

(*b*) *Nitrogenous compounds*

Two interesting lectures on amino-acid analysis and the structure of proteins have been given by Chibnall (99, 100). His theoretical considerations are mainly based on data for β-lactoglobulin and casein. Chibnall, Rees & Williams (101) have recorded values of 15·58 and 15·73% for the N content of β-lactoglobulin and casein respectively. They consider that many recent figures are low due to inaccuracies in analytical procedure. Figures for the amino-acid content of β-lactoglobulin are given in the following papers: aspartic acid (99, 102, 103) glutamic acid (99, 103), arginine (99, 102, 103), histidine (99, 102, 103), lysine (99, 102, 103), valine (102, 104), phenylalanine (102, 104) leucine (102, 104), *iso*leucine (102, 104), alanine (104), proline (104), cystine (102), tyrosine (102), tryptophane (102) threonine (102, 105) and serine (105). Corresponding data for casein are found in the following papers: valine (104, 106), phenylalanine (104, 106), leucine (104, 106), *iso*leucine (104, 106), alanine (104), proline (104) lysine (107, 108), histidine (100, 107, 108), arginine (104, 107, 108), threonine (105), serine (105, 109), tyrosine (108, 110) and tryptophane (108, 110). Gordon, Semmett, Cable & Doherty (108) have given some amino-acid data for α- and β-casein. Dunn & Rockland (111) have tentatively proposed a figure of 3·00% of histidine, an amino-acid which is not appreciably destroyed during acid hydrolysis, as a standard of reference for all other amino-acids in casein. The amino-acid composition of casein and lactalbumin of human milk has been studied by Williamson (112). Values for the valine, leucine, *iso*leucine and phenylalanine in the protein of samples of dried skim milk and dried whey have been provided by Schweigert (106). Kugenev (113) observed variations in amino-acid composition when comparing the casein obtained from the milk of individual cows.

Anantakrishnan & Lahiry (114) have given the proximate analysis and nitrogen distribution of samples of casein and lactalbumin isolated from the milk of the ass. The course of tryptic and peptic digestion of a casein sample did not differ materially from that of the casein of cows' milk. Waisman & Petazzo (115) have made a study of the daily variations in the detailed nitrogen distribution of human milk. Variations of up to 100% in the casein content were observed from day to day. Karabinos & Dittmer (116) have isolated hippuric acid from the non-protein nitrogen fraction of cows' milk. Willstaedt, Borggard & Lieck (117) have reported values of 40 and 56 mg./100 g. for the choline content of butter and cheese.

By dry grinding water-insoluble casein for four periods of 48 hr. each, Cohen (118) was able

to separate it into one water-insoluble and four water-soluble fractions. Analysis of the fractions indicated the breakage of specific linkages in the casein molecules. The sulphuric acid hydrolysis of casein has been studied by Frost & Heinsen (119) and Schein & Berg (120). The rennet action of pancreatin on casein has been studied by Feigenbaum (121), who considers that the enzyme acts by weakening the protective colloid component. Hoover & Kokes (122) found that when casein was hydrolysed by papain to a stage at which 1% of the peptide bonds had been split, the usual β-casein peak could no longer be detected in electrophoretic examination; the boundary of the α-casein was diffuse. Winnick (123) has observed that in the enzymatic digestion of casein, the initial products are protein molecules containing an average of five to seven amino-acid residues. Further action of carboxypeptidase on protease digests is considered to result in the breaking off of amino-acid groups from the end of the molecule rather than the production of smaller peptide units. Cheesman & Ehrensvärd (124), following new approaches to the investigation of the structure of protein molecules, suggest that aromatic NH_2 residues tend to be concentrated in a definite region of the casein molecule. Maass (125) has observed that the action of pectin plus pectase, in the presence of calcium ions, on milk or whey leads to precipitation of the proteins. The action of pepsin on β-lactoglobulin has been thoroughly investigated by Haugaard & Roberts (126).

Studying the heat-coagulation time of milk samples in sealed glass tubes at temperatures between 110 and 160° C., Cole & Tarassuk (127) found considerable variation in the coagulation time of different milks at a given temperature. In the coagulation of milk by rennet, Sandelin (128) found the precipitation of casein incomplete. He investigated the influence of several factors on the proportion of the casein remaining in solution, and found the concentration of ionic calcium to be the most critical factor. Sørensen & Sørensen (129) have separated the whey proteins into five fractions (three of which may be identical). Details of the separation process and physical properties of the fractions are given. Harland & Ashworth (130) have studied the heat denaturation and precipitation of soluble proteins in heated skim milk. Dills & Nelson (131) have obtained from cows' milk a protein similar in copper content to the copper-bearing proteins isolated from various plant and animal tissues.

(*c*) *Lactose*

Pino (132) has observed a reduction in the lactose content of cows' milk during periods of work.

Decker & Reid (133) have reviewed earlier work on the factors affecting lactose crystallization in ice cream, condensed milk and processed cheese. By polarimetric observation, they have shown that after 3–4 hr. hardening at -8 to -12° F. lactose crystals appear; with periods up to 24 hr. the number and size of crystals increase markedly, but on prolonged storage a decrease in number and sometimes size occurs. Aas (134) has determined polarimetrically that the milk sugar in whey cheese is present wholly as α-lactose. The effect of stirring the cheese during cooling after the evaporation process, in promoting fine crystallization, is illustrated by photomicrographs. Leviton (135, 136) has studied in great detail the adsorption of riboflavin on lactose crystallizing from whey concentrates. Adsorption takes place only when the riboflavin exceeds a certain threshhold value determined by the degree of supersaturation of lactose and by the temperature. When lactose crystallization is complete adsorption increases linearly with the initial concentration of riboflavin.

(*d*) *Minerals*

Observations on the calcium content of bulk samples of whole milk have been reported by Semmons & McHenry (137) and Robinson & Hlynka (138). Seekles & Smeets (139) found no relationships between the occurrence of the 'Utrecht abnormality' (coagulation of fresh milk on boiling) and soil type or season, but observed that it was favoured by over- or under-feeding. The fault could be eliminated by the addition of sodium citrate to the milk. The administration of sodium citrate to the cow orally or subcutaneously was also sometimes effective. Sandelin (140) has shown that the solubility of calcium phosphate in aqueous systems is influenced by the concentration of citric acid, the pH of the solution and the presence of sodium or potassium and chlorine ions.

He determined the solubility in a 0·2% solution of citric acid with contents of sodium or potassium and chlorine ions and a pH similar to that of milk. When the phosphate-citrate solution was warmed, both calcium phosphate and calcium citrate were precipitated and the solution became more acid, but on cooling a portion of the precipitate redissolved. This may explain why the action of rennet on heated milk becomes more effective when the milk is cooled and kept for some time. Basu & Mukherjee (141) have determined the partition of phosphorus, between the inorganic, ester, lipid and casein fractions, in samples of cows', sheep's, goats', buffaloes' and human milk. Sandelin (142) has found 36–40% of the trichloracetic acid-soluble calcium, 55–62% of the apparent inorganic phosphorus and 68–88% of the true inorganic phosphorus to be retained by the whey fraction of milk after rennet coagulation. The effect of pre-heating and precipitating conditions on these values is recorded.

White, Clifford & Calvery (143) observed increases in the level of lead in the milk of cows that had been given sublethal doses of red lead, while Stas (144) has shown that considerable amounts of lead in milk and buttermilk may result from their corrosive action on the solder and tinned surfaces of containers. The iron, copper and cobalt contents of the colostrum of Jersey, Holstein and beef cows have been determined by Luecke, Duncan & Ely (145). The values for iron and copper are higher than the known values for milk. Cobalt is present only in traces (0·001–0·012 p.p.m.). No marked breed differences were observed. Values for the iron content of cows' milk, buttermilk and human milk have been given (146–149). Itzerott (149) has tabulated values for the range and average of copper and iron in samples of fresh milk and other dairy products. The contamination of milk during handling and processing is illustrated, and the maxima compatible with good keeping quality of the products have been determined. It was observed that after the separation of milk the two metals were distributed in proportion to the protein and water content of each fraction. When cream was churned, they concentrated on the fat-globule surface in the form of an adsorbed metal-protein complex. Data for the copper, lead and zinc content of samples of whole milk, human milk, butter, dried milk, various cheeses, and milk proteins have been reported by Schwaibold & Lesmüller (150). An increase in the average cobalt content of milk to approximately four times the normal level of 0·6μg./l. was noted by Archibald (151) with cows receiving a daily supplement of 500 mg. of cobaltous acetate. Holland & Ritchie (146) have found that milk contains manganese in traces. Venn, McCance & Widdowson (152) have obtained Fe values varying from 235 to 282 μg./100 g. for sows' colostrum and from 153 to 240 μg. for sows' milk. It was confirmed that the administration of iron to lactating sows does not increase the iron content of their milk.

(*e*) *Miscellaneous*

Values for the phospholipid and lecithin content of milk and other dairy products are recorded (153, 154). Kahane & Lévy (155) have made a detailed study of the choline content of milk, separating it into insoluble and soluble choline and subdividing the latter into free and soluble combined choline. Variations in these fractions and the distribution of the fractions between the aqueous and fatty phase of milk have been studied.

Townley & Gould (156, 157) consider that the soluble proteins and the substance associated with the fat of milk are the source of the heat-labile sulphides. Sulphide liberation is shown to be influenced by the stage of lactation of the animal and the pH of the milk. The effect of the addition of various chemicals has also been determined. Gould & Frantz (158) have shown that the formic acid content of milk heated to 116° C. increased with time.

Howell, Cave, Heller & Gross (159) have detected D.D.T. in the milk of cows sprayed with D.D.T. preparations. However, even when animals were sprayed with doses much larger than would normally be used, the milk gave negative results in biological tests for toxicity.

(*f*) *Enzymes*

Phosphatase

Caputto & Marsal have prepared purified extracts from autolysed bovine mammary glands (160) and have studied some of their physical and chemical properties (161). Studies of the activation and inhibition of milk phosphatase by various salts have been reported by Kelly (162), and by Massart & Vandendriessche (163), who suggest that milk phosphatase is a heavy metal (zinc) proteid. According to Sizer (164), changes in the oxidation-reduction potential do not affect the activity of milk phosphatase unless the E_h value is increased to more than +400 mV. Bever (165) and Bever & Straub (166) have drawn attention to the similarity of the mechanism of thermal destruction of phosphatase, tubercle bacilli and the agglutinins responsible for the creaming of milk to that involved in the heat denaturation of the soluble-milk proteins. Their mathematical treatment has been modified by Stijgeren (167). Hartmann & Andersen (168) have investigated the kinetics of phosphatase hydrolysis, and have drawn attention to the advantages of *p*-nitrophenyl-phosphate as a substrate, the yellow colour of the liberated *p*-nitrophenol serving as a direct indicator of the progress of the hydrolysis. Sanders & Sager (169) have measured the residual phosphatase activity of milk and dairy products subjected to varying, controlled times and temperatures of heating. On the basis of preliminary experiments with lipoid solvents, Sjöström (170) has suggested that milk phosphatase is linked with the proteins rather than the lipoids of the fat-globule membrane. According to Guittonneau, Chevalier & Jarrousse (171), milk contains two pyrophosphatases with maximum activity at pH 4·2 and 7·7 respectively. They show little activity in milk, more in cream, and most in buttermilk and the aqueous phase of butter.

The value of the phosphatase test in the control of the efficiency of pasteurization has been discussed by several authors (172–176). Burgwald (177) has surveyed the extent to which the test is used in North America. Replies to a questionnaire from more than 200 laboratories showed that an average of 2·7 % of about 200,000 samples failed to pass the phosphatase test.

A new phosphatase test, based on phenolphthaleinphosphate as the substrate, has been described by Stiven (178). Enzyme activity is estimated by the colour of phenolphthalein liberated during incubation for 24 hr. Numerous modifications of the Scharer test have been proposed (179–184). Following an investigation of the sources of errors in the Scharer test, Hartmann (181) suggested a more elaborate test on a casein-free milk filtrate. Later, his colleagues (182) developed a highly accurate technique based on Aschaffenburg & Neave's modification of the Scharer technique. Horwitz (183) modified the Scharer test after a careful investigation of the underlying fundamental and technical principles. An improved technique given by Sanders & Sager (184) is applicable not only to milk, but—with appropriate adjustments—also to most other dairy products.

Difficulties likely to arise when the phosphatase test is applied to sterilized milk have been discussed by Aschaffenburg (185), who has proposed a simple turbidity test based on the absence of heat-coagulated proteins. Andersen (186) and Thomé (187) have shown that the phosphatase test is applicable to whey. Brown & Elliker (188) observed high phosphatase readings in applying the Kay-Graham test to neutralized sour cream, stored at temperatures above 15° C., to which salt had been added after treatment in a Vacreator. Their results resemble earlier findings by Wiley, Newman & Whitehead (*J. Coun. sci. industr. Res. Aust.* **14**, 121, 1941). Barber & Frazier (189) have shown that the reversion to a positive phosphatase test which may occur in pasteurized cream after storage at low temperatures is caused by the development of phosphatase by certain strains of *Bacillus cereus* and *B. mesentericus*. The bacterial enzyme was more heat-resistant than milk phosphatase.

Several modifications of the Scharer technique have been proposed for Cheddar cheese (184, 190, 191). That of Sanders & Sager (184) was tested together with the Kay-Graham technique in a collaborative experiment between thirteen laboratories, on which Gilcreas (192) reports. The technique of Sanders & Sager proved satisfactory, whilst the use of the Kay-Graham technique was abandoned because protein breakdown products caused high colour readings. The usefulness of a technique developed by Horwitz (183) for soft cheese, such as cottage cheese, has also been demonstrated in

a collaborative experiment(193). Details for the application of the phosphatase test to a great variety of dairy products have been given by Sanders & Sager(194).

Rennin

The preparation of crystalline rennin has been reported by Hankinson(195,196) and Berridge(197,198). Hankinson & Palmer(199) have made a physico-chemical study of the reduction in the stability of calcium and sodium caseinate sols caused by the addition of rennin. Stijgeren(167) has demonstrated the quantitative presence of the coagulating principle in the whey obtained after renneting. He made a precise study of the inactivation of renneting power between 50 and 60° C., and calculated complete thermal destruction after heating to 63° C. for 10 min. or to 81° C. for 1 sec. The effect of changes in the composition of the casein complex of milk on renneting time has been investigated by Pyne(200). Moriyama(201) observed a destruction of activity of the monomolecular reaction type when an acidified solution of commercial rennet was neutralized. He has drawn attention(202) to some physical and chemical similarities between rennin and certain viruses. Moriyama & Ohashi(203) studied the inhibitory action of hydrogen sulphide in the presence of salts of heavy metals. According to Mazé(204), addition of increasing quantities of sodium chloride to skim milk leads to corresponding increases in clotting time. Mazé & Mazé Jnr.(205) observed that dilute solutions of mineral salts affected the clotting time when added to milk, but not when added to rennet. Vilegzhanin(206) reports a detailed compositional study of milk from a Friesian herd grouped into slow-, moderate- and quick-clotting milks. The slow-clotting milks were characterized by low ionic calcium levels. A scheme of determining the clotting power of commercial rennet extracts by comparison with that of a known stable extract has been suggested by Tapernoux(207).

Lipase

Peterson, Johnson & Price(208) have modified the method of Reder for the determination of milk lipase. Further studies of lipase action have been reported by the Cornell workers(209–211). They found rapid destruction of the enzyme in butter held at 155° F. or above (209). Lipolysis was found in samples of unsalted sweet cream butter stored for more than 1 year at 30–32° F.; inhibition was noted in samples similarly held at 5° F. or below(209). Lipolysis of milk on storage at low temperatures was at a minimum when the milk had been cooled in a matter of seconds, rapid cooling being particularly essential between 20–25 and 0° C.(211). The relationship between fat acidity and subjective flavour scores was also investigated(210). Kay (212) has drawn attention to the great light-sensitivity of milk lipase.

Tarassuk & Henderson(213) observed a marked decrease of surface tension in those milks of cows on winter feed which rapidly developed a rancid flavour, and Hlynka & Hood(214) found a low, but significant, correlation between surface tension and rancid odour in evening milks stored overnight at 5° C.

Kelly(215) has studied the hydrolysis of a number of pure substrates by the lipase contained in the non-fatty fraction of milk. Results with tributyrin gave the best agreement with subjective tests for rancidity. The presence of pitocin stimulated the lipolytic activity(216). Gould(217) found no oxidative changes in the fat of homogenized milk on lipolysis, and no inhibition in the presence of copper or formalin. These differences from the behaviour of raw milk suggest the existence of two milk lipases. Lipolytic activity in homogenized milk was derived from material in the skim-milk fraction. Gould(218) has also studied the action of lipase on various oils and fats homogenized in skim milk. Jack, Tarassuk & Scaramella(219) observed that butter made from cream containing active lipase stored well at 20–22° F. when the cream was pasteurized for 30 min. at 150° F. or above. If the cream was held for 3 days at 40° F. prior to heat treatment, an inferior butter resulted. Hlynka, Hood & Gibson(220) made Cheddar cheeses from milk containing varying amounts of pancreatic lipase with or without additions of copper. Presence of the metal had no effect on the flavour of the finished cheese though it improved the flavour during manufacture. Babel & Hammer(221) found no correlation between the degree of rancidity and the fat acidity of Cheddar cheese made from pasteurized milk to which rennet extract or mulberry juice had been

added as a source of lipase. According to Lythgoe & Rosenthal(222), goats' milk contains less lipase than cows' milk. 'Goaty' flavour may be due to the relatively high caprylic acid content of the fat of goats' milk.

Other enzymes

A study of highly purified milk peroxidase(223) has revealed significant differences from plant peroxidases. Theorell & Paul(224) have crystallized the enzyme. It has a molecular weight of 93,000(225). Fischer(226) has studied the reactivation of peroxidase in heated milk. The reactivated enzyme was less heat-resistant than the original one. An explanation of the mechanism of reactivation is put forward. Quantitative methods for the determination of milk peroxidase have been described by Fischer(226), Schwarz & Fischer(227), Kreker(228) and Diemair & Häusser(229). The value of the peroxidase test for controlling the efficiency of pasteurization has been studied by several authors(227, 228, 230), the results being, in general, disappointing. Schwarz & Sydow(231) found guaiacol (2%) a suitable substitute for other peroxydase test reagents which were no longer available. Schönberg & Milbradt(232) have demonstrated that leucocytes and particles of pus and of protein protect peroxydase against thermal destruction. Schellhase(233) applied various forms of the peroxydase tests to milk of different animals, and noted marked differences in response.

The amylase test as a means of assessing the efficiency of pasteurization has been studied by several authors(227, 228, 234).

Worden(235) and Polonovski, Neuzil, Baudu & Polonovski(236) have provided good evidence of the linkage of the xanthine oxydase of milk to the fat-globule membrane. Cooling of milk to low temperatures releases the enzyme and results in increased enzyme activity. Polonovski *et al.* have effected this release in warm milk by the replacement action of surface-active materials. Araujo(237) has reported the curious observation that heated milk gives a positive Schardinger reaction after addition of ascorbic acid in artificial light, but not in the dark or in daylight. The possible role of xanthine oxidase in biological iodinations has been studied by Keston(238).

Warner & Polis(239) have presented strong presumptive evidence that an enzyme is responsible for the proteolysis of casein. The proteolytic factor could not be separated from casein, but was concentrated to about 150 times its original activity.

Vilstrup & Bielefeldt(240) have suggested that a cacao-bean enzyme is responsible for the coagulation of milk which occurs occasionally when mixtures of milk and cocoa or drinking chocolate are heated.

III. Physical chemistry

Proteins

The application of modern physico-chemical methods, such as the use of the ultracentrifuge and of the electrophoresis apparatus, has led to welcome advances in the knowledge of milk proteins.

Casein

Warner(241) observed two main peaks in the electrophoretic pattern of casein, and developed a method for the isolation of these fractions (α- and β-casein). They tend to form complexes, as was also observed by Krejci, Jennings & Smith(242). A splitting of the α-casein into two components was observed electrophoretically by Nitschmann & Lehmann(243) after acid casein had been treated with rennet in calcium-free barbitone buffer. According to Mellander(244), human and cows' milk casein have very similar electrophoretic patterns.

An ultracentrifugal study of calcium-caseinate solutions(245) showed that the relationship between calcium and casein can be expressed in terms of the law of mass action. Klotz(246) has shown that this law can be invoked to calculate the equilibrium constants for the interaction of calcium with casein.

The complex problem of the state of the casein in milk has been investigated by several authors. On the basis of a detailed study of the composition and properties of milk casein, Eilers(247) has

developed a concept of hydrated micelles in which tricalcium phosphate is bound up with calcium caseinate. The conclusions reached by other Dutch workers as the result of experiments with supercentrifuged skim milk (248) or of adsorption studies (249), are largely in agreement with the views of Eilers. A study of the composition of the casein complex by Ramsdell & Whittier (250), in which the supercentrifuge was used for isolation, has also provided evidence that the phosphate is present in the form of the tricalcium salt. Sandelin (251, 252) prefers an orthodox chemical to a colloidal viewpoint, assigning a dominant role to the calcium ions. Küntzel & Doehner (253) have developed a far from conventional picture of spherical micelles composed of hydrated aggregates and disperse particles in true solution, a picture mainly derived from a study of the swelling behaviour of milk casein. That the casein particles are spherical is also indicated by experiments on the depolarization and light-scattering of separated milk conducted by Lotmar & Nitschmann (254). In a general study of the viscosity changes during gradual degradation of proteins, Jirgensons (255, 256) found casein to behave like a typical globular protein, showing on mild treatment an increase in viscosity thought to be due to the unfolding of the molecules.

Studies of the spreading of casein, and of the nature of the surface films, have been reported by Rao, Doss & Rao (257, 258). Erdös (259) and Laki (260) have measured the effect of various salts on the isoelectric point of casein. Jirgensons (261) studied the stability of sodium caseinate dispersed in mixed solvents, and the effect of salt additions. A study of the effect of dry heat on various proteins by Mecham & Olcott (262) contains data for commercial acid casein. Nitschmann & Lehmann (263) found that in neutral solutions of sodium caseinate, derived partly from rennet and partly from acid casein, calcium ions precipitated only the casein derived from rennet.

A study of the chemical and nutritional differences between the casein of human and cows' milk has been reported by Mellander (264). Olcott & Fraenkel-Conrat (265) have tried to improve the suitability of proteins as plastics by increasing their water resistance through chemical modification. They succeeded, for instance, in reducing the water absorption to as little as 1·6 % in a sample of casein treated with phenyl*iso*cyanate. Mellon, Korn, & Hoover (266) studied the role of free amino-groups in the absorption of water by casein by partial elimination of these groups through benzoylation.

Reaction of casein with formaldehyde

The reactions of casein with formaldehyde, of particular importance to the plastics industry, have been the subject of much study. The effects of addition of formaldehyde to amino-acids and proteins in general, have been reviewed by French & Edsall (267). Nitschmann and his co-workers have presented a series of papers in which they deal with the analytical problems of determining formaldehyde bound by casein (268–270), with the binding of aqueous and gaseous formaldehyde (272), and with the swelling power of hardened casein (273). High-temperature treatment (70° C.) increased the uptake of formaldehyde, but did not improve the hardening of casein (270). Studies with casein preparations in which the amino-groups had been blocked by deamination (274) or acetylation (275) indicated that formaldehyde acts on casein by forming a CH_2 bridge between lysine amino-groups and peptide groups. The mechanism of the binding of formaldehyde by casein has also been discussed by Carpenter & Lovelace (276), who have pointed out that more aldehyde is bound than is consistent with the assumption that only amino or amide groups form linkages. They have shown that the uptake of formaldehyde by acid and rennet casein and also by deaminized casein (277) can be described in terms of the adsorption isotherm. Working with casein, desamido-casein and zein, Wormall & Kaye (278, 279) reached the conclusion that formaldehyde is linked to amino-groups with proteins at or near their isoelectric point, and to amide groups at lower pH. Fraenkel-Conrat, Cooper & Olcott (280) have criticized existing theories on the nature of the interaction of proteins and formaldehyde. Their own results have indicated that the primary amino- and amide groups play a dominating part, whereas the secondary amide groups of the peptide chain and the phenolic groups are hardly involved.

β-lactoglobulin

The molecular weight of the crystalline protein has been determined by a variety of methods. From a detailed amino-acid analysis, Brand & Kassell(281,282) calculated a value of 42,000. From osmotic pressure measurements, Gutfreund(283) obtained 38,000 and Bull & Currie(284) 35,050. From mono-layers spread on concentrated salt solutions, Bull(285) calculated a value of 44,000, later amended to 34,300(286). Films spread in the absence of heavy metal ions led to a value of about 17,000, suggesting a cleavage of the molecule into two equal parts.

Crystallographic data for β-lactoglobulin have been provided by Fankuchen(287). McMeekin & Warner(288) determined the hydration of crystals to 0·83 g. of water per g. of anhydrous protein. Data for the degree of hydration and asymmetry of crystalline β-lactoglobulin in aqueous solutions have been given by Gorin & Moyer(289) and by Briggs & Hanig(290). Electrophoretic measurements by Li(291) showed that crystalline β-lactoglobulin did not migrate homogeneously at some pH values, a finding which suggests that the crystalline protein may not be a single substance. Other work with solutions of crystalline β-lactoglobulin includes a study of the hydrogen-ion dissociation curve(292), solubility studies(293–295) showing that the addition of certain amino-acids(294) and of ovalbumin(295) led to an increase in solubility, a study of the heat denaturation in neutral solution(296) showing that the first effect of heating is the formation of approximately fourfold molecular aggregates, and a study of the surface activity(297) of solutions of varying ionic strength, pH and age. Miller(298) has made an investigation of the hydrolysis of crystalline β-lactoglobulin by crystalline trypsin and chymotrypsin.

A study of the adsorption of water vapour by proteins by Bull(299) includes data on β-lactoglobulin. The results form the basis for theoretical considerations by Bull himself and by Pauling(300).

Immune proteins

The use of physico-chemical methods has led to marked advances in our knowledge of the important protein fraction of bovine colostrum which contains the immune antibodies so essential to the new-born calf. E. L. Smith(301) has shown that the immune activity is associated with an electrophoretically slow-moving fraction which may account for as much as 55% of the nitrogen content of colostrum. The immune lactoglobulin contained euglobulin and pseudoglobulin in the ratio 4:3. Smith & Coy(302) have measured the ultra-violet absorption spectra of various immune proteins of the cow, horse and human. Smith(303) found that in ordinary whey, immune lacto-globulins are present to the extent of *c.* 10% of the total proteins, and that they may be identical with those of colostrum. Deutsch(304) found characteristic species differences in the electrophoretic patterns of the whey proteins prepared from the milk of the cow, goat, sow, ewe, mare and human. A high concentration of slow-moving globulins was observed in colostral wheys. The marked changes in the electrophoretic pattern of cows' milk during early lactation have been demonstrated and discussed by Grönwall(305). Hansen, Phillips, Williams & Smith(306) found in electrophoretic experiments that the serum γ-globulin, very low at birth, had increased markedly within 18 hr. after calves had received colostrum, and that in calves reared on skim milk the γ-globulin level was still low after 8 weeks.

General properties

Surface activity, foaming

Aschaffenburg(307) applied a dilution technique to measurements of the surface activity of the protein constituents of milk. He found that, in addition to casein, a fraction of low molecular weight, σ-proteose plays an important part. This fraction has been isolated. Examination in the ultracentrifuge (addendum by Ogston) showed it to be somewhat heterogeneous. The role of the surface-active proteins in the foaming of milk and milk products has been the subject of a detailed study by El-Rafey & Richardson(308). They succeeded in duplicating the foaming properties of natural systems with mixtures made up from isolated protein fractions. They showed that the minimum in the foam stability of separated milk or whey at temperatures near 25° C. is due to the presence of fat particles, and disappears after supercentrifugation. The importance of foaming

properties in the preparation of whipped cream of low fat content has been discussed by Sommer (309).

Fat-globule membrane

An authoritative review on the structure and properties of the fat-globule membrane has been provided by Palmer (310). In experiments described in the same paper, it is shown that the addition of fractions rich in membrane protein, e.g. buttermilk, cause the most marked reduction of the interfacial tension butterfat/water at 40° C. Previous data on the preparation and analysis of membrane material have been amplified by Jenness & Palmer (311). They determined the protein: phospholipid ratio in washed cream and in the products obtained after churning. According to Sandelin (312), the fat-globule membrane consists of an inner phospholipid layer associated with the fat and an outer protein layer which is only loosely held. Sandelin determined the concentration of the protein in various milk products, and analysed the isolated material. A later study by Sandelin (313) deals with the mechanism of adsorption in emulsions prepared from butterfat and a variety of emulsifying agents. With agents of a protein nature, emulsions were stabilized by the adsorption of kations, whereas with all other agents anionic adsorption occurred.

Mechanism of creaming

Skelton (314) reports experiments with concentrates of the fat-globule agglutinin of milk. Its activity was not impaired when the casein was removed by renneting. Dunkley & Sommer (315) have isolated the agglutinin which was found to be of a euglobulin nature. Its mode of action was observed to be very similar to that of bacterial agglutinins which are, in general, of a globulin character. The report includes a review of the existing theories of the mechanism of creaming. In a study of cream rising in milk, Skelton & Sommer (316) found some evidence of a wave-like progress of the fat rising in a column of milk after the initial period of rapid creaming. Peters & Trout (317) have demonstrated that leucocytes are attracted by the fat globules of milk. In experiments (318) in which washed leucocytes were added to systems made up from washed cream and heated whey at varying pH values, maximum creaming occurred at pH 4·3, the isoelectric point of the fat globules.

According to Straub & Bever (319), their creaming test (166) can be used to differentiate between under-pasteurization and presence of raw milk in H.T.S.T.-pasteurized milk which gives a positive phosphatase test.

Miscellaneous

A spectroturbidimetric study of milk proteins has been made by Leviton & Haller (320), who have treated their data on diluted skim milk, homogenized milk and aqueous dispersions of alcohol-precipitated proteins in terms of Mie's theory of the scattering of light by particles. A note by Mocquot (321) on 'bound water' concerns observations made on rennet curd in equilibrium with various sugars. The percentage of 'bound water' rose with increasing molecular weight of the sugars from 24 to 28% for C_6 to 59% for C_{18}. Occhialini (322) has recorded the changes in the E_h and pH values of milk at intervals of 2 hr. for 48 hr. after milking.

Lyons (323) has described a direct microscopic method for the determination of the size distribution of fat in diluted milk or cream. He has used the method to demonstrate the changes in size distribution due to mechanical treatment, e.g. in pasteurizing plants of different design. Mulder (324) has demonstrated that, even after powerful centrifugation, some true fat remains dispersed in buttermilk, mainly in colloidal form. The possible value of the dielectric constant as a butter characteristic has been considered by Mohr & Hennings (325). Determination of the constant did not provide a reliable measure of the moisture content. The authors utilized the technique successfully to follow the phase reversal during the manufacture of Alfa butter.

IV. Processing and manufacture

Homogenization

Trout & Scheid (326) have found a minimum temperature of 100° F. necessary for the satisfactory dispersion of fat during homogenization. Lipolysis was most pronounced at homogenization temperatures of 80–120° F. and much less marked at 140° F. At 40° F. no lipolysis occurred, but efficient homogenization was impracticable. Babcock (327) observed that the reduction in the curd tension of milk increases with increasing homogenization temperature and homogenizing pressures up to 2000–2500 lb., and with increasing fat content of the milk. The *in vitro* digestion of homogenized milk proceeded at a more rapid initial rate than that of raw milk, probably because of the increased surface area of the curd, but no differences in digestibility were found. Peters & Trout (328) have stressed the need for clarification of homogenized milk if sediment formation is to be avoided. Efficient clarification is possible even with the fat in the solid state and is better carried out before homogenization because fragmentation of leucocytes in the homogenizer (329) makes their removal by clarification difficult if not impossible. A modified Tromsdorff test developed by Peters & Trout (330) was applied unsuccessfully to the selection of potentially sediment-free milk for homogenization.

The method for testing the efficiency of homogenization laid down by the U.S. Public Health Service in 1939 has come in for criticism from several quarters (331–335). The requirement that in homogenized milk stored for 48 hr. the fat percentage of the top 100 ml. should not differ from that of the remainder of a quart by more than 5% has been shown to be too stringent, and the U.S. Public Health Service tolerance has been increased to 10% in 1943. The technique of testing has been criticized as being too vague, and more definite and precise conditions of testing have been proposed. Doan & Mykleby (334) have obtained favourable results with a modification of a method proposed by Farrall, Walts & Hanson (337) which is based on microscopic counts of the fat-globule size. Yet another method has been described by Snyder & Sommer (338). This is based on stepwise dilution and centrifugation in Babcock tubes giving results in line with those of the U. S. Public Health Service technique.

Heat treatment

Brasch & Huber (339) have described a process of milk sterilization based on exposure to electron irradiation for short periods (1×10^{-6} sec.).

Gould (340) has reviewed the chemical changes observed in milk subjected to high-temperature heat treatment. Harland, Jenness & Coulter (341) studied the effects of exposure to high temperatures for prolonged periods on skim milk and various aqueous buffered systems of milk constituents, in an attempt to determine the mechanism of the browning reaction of milk products. A lactose-caseinate system gave the darkest colour. Lactose alone caused some browning, while casein alone produced a slight but definite pink coloration. On the basis of potentiometric Sørensen formaldehyde titrations of skim and dialysed milk and milk-like systems before and after autoclaving, Kass & Palmer (342) have concluded that browning does not consist of a simple aldose-casein condensation but rather of a polymolecular reaction accompanied by dehydration of sugar. Anantakrishnan *et al.* (37) have determined the effect of various heat treatments on the physical and chemical properties of milk from Indian cows and buffaloes.

Guittonneau, Bejambes, Veisseyre & de Lamothe (343) observed an increased precipitation of phosphate and a more marked reduction in acidity with milk pasteurized in a stream of oxygen as compared with normally pasteurized milk. They suggest that the lowering of acidity during ordinary pasteurization is a contributing factor in increasing the keeping quality.

Josephson (344) and Josephson & Dahle (345) have studied the influence of other milk constituents on the effect which heat treatment has on the keeping quality of butterfat. While the heating of butterfat alone reduced the induction period, the heating of butterfat with dried skim milk, or the heating of butter, at temperatures of 300–400° F. for 10 min. increased the induction period. Ewbank & Gould (346) obtained an increase in the stability of butterfat by flash pasteurization

of the cream at 85 or 90·6° C. previous to churning. The increased stability was attributed to the formation of reduced sulphide compounds.

Lea (347) has attributed the improved keeping quality of dried whole milk obtained from liquid milk preheated to a high temperature to the anti-oxidant properties of sulphydryl compounds formed from protein during the heat treatment. Findlay, Higginbottom, Smith & Lea (348) have studied the effect of preheating milk at temperatures of 160–200° F. for 2 days prior to spray-drying on the keeping quality and the physical, chemical and bacteriological properties of the powdered product. The original should be consulted for details of the large number of observations. White, Smith & Lea (349) have observed the effect of a high preheating temperature with and without ethyl gallate on the storage life of spray-dried whole-milk powder. It is tentatively concluded that for normal use a powder prepared from a milk sterilized with ethyl gallate and preheated to 160° F. for 5 min. is not preferable to one made from milk preheated to 190° F. Webb, Bell, Deysher & Holm (350) have determined the effect of the time (0–16 min.) and temperature (95–150° C.) of the forewarming of milk before concentration on the rate of heat coagulation of milks concentrated to various solids content. High temperature-short time forewarming increased the heat stability of samples and permitted sterilization without coagulation of samples concentrated to a higher solids content. With optimum forewarming, usually 120° C. for 3–4 min., drying up to 35–37 % of solids was practicable and without adverse effect on the colour of the evaporated milk (351). Webb & Bell (352) have considered the effects of a second heat treatment after concentration on heat stability.

Freezing

Babcock, Roerig, Stabile, Dunlap & Randall (353) have studied the effects of low-temperature storage on homogenized milk. Samples stored at −32·8 and −40° C. for 115 days showed no loss in quality; samples frozen at −10° C. and then stored at −32·8° C. kept almost as well, but samples stored at a higher temperature than that of the original freezing developed oxidized flavours and showed signs of physical separation. The same authors (354) observed that frozen homogenized milk, stored under a variety of freezing and holding conditions, showed an increase in all solid constituents in the lower fractions. Doan & Warren (355) have investigated the gradual and progressive flocculation of protein material in concentrated skim milks kept in frozen storage. They attribute this precipitation to the salting-out effect of minerals concentrated in the unfrozen water. Freeman, Mull & Fouts (356) have compared various methods of processing and treatment of cream prior to frozen storage. Providing the copper content was low, cream stored for 7 months at 0° F. without the addition of anti-oxidants still produced a satisfactory ice cream. Mohr & Eysank (357) have studied the destabilization of the fat emulsion in cream during frozen storage. The degree of instability depended largely on the time of storage and was relatively independent of such factors as previous heat treatment and mode of freezing. Consequently, they suggest that factors other than the disruptive influence of ice formation, possibly crystallization of fat, may contribute to the loss of stability.

Gas packing and storage

Greenbank, Wright & Deysher (358) observed a marked improvement in the keeping quality of gas-packed whole-milk powder when air was replaced by nitrogen. Lea, Moran & Smith (359) have shown the critical effect of the oxygen concentration in the head space on the keeping quality of gas-packed dried milk or butterfat. With both products, tallowiness could be prevented indefinitely by packing gas-tight containers in a nitrogen atmosphere containing not more than 0·5–2 % of oxygen. Details of the gas-packing technique are given. Difficulties are considered which result from the desorption of air in spray-dried powders. Coulter & Jenness (360) have found 'double-gassing' and vacuum holding effective in overcoming desorption. These authors and Hetrick & Tracy (361) have dealt with factors in the spray-drying of whole milk and the gas-packing of the powder which determine the oxygen content of the resulting product. It is suggested by Greenbank, Wright, Deysher & Holm (362) that taste and smell give a better indication than peroxide values of the quality of gas-packed samples. They have observed the development of

tallowiness without an accompanying increase in peroxide value, possibly because the rates of formation and destruction of peroxide are equal.

Thiel (363) reports that the storage of whole-milk powder as compressed blocks in cellophane-like wrappings or in the form of compressed tablets is not effective. Doob, Willmann & Sharp (364) found that high osmotically held moisture, low pH and low lactose content tended to induce formation of brown colour in dried wheys. During storage of dried whey, and also of dried skim milk, browning was accelerated by high relative humidity and temperature. Webb, Deysher & Hufnagel (365) have studied the physical changes which take place during the storage of evaporated skim and whole milk. The viscosity of all samples decreased during storage to a basic level, the logarithm of the time taken to reach this level being related linearly to storage temperature. The magnitude of the change varied markedly. Protein flocculation was least in milk of high-fat content. Homogenization helped to retard protein separation. Small hard crystals which formed in some samples consisted largely of calcium citrate. Development of colour in samples sterilized at 225–255° F. increased linearly with increases in time and temperature. Colour development during storage was directly related to the logarithm of the time of storage. The combined deposits from a large number of tins of evaporated milk have been analysed by Gould & Leininger (366). The composition of the crystals indicated that the deposit consisted mainly of the tetrahydrate of tricalcium citrate.

Miscellaneous

Romani (367) considers hydrogen peroxide to be a suitable preservative for milk supplies from outlying farms.

Gould, Weaver & Frantz (368) showed that, under ordinary conditions of storage, lactic acid formation in evaporated milk was negligible and of no practical importance, whereas marked increases in formic acid occurred.

Loftus-Hills (369) observed a reduction in the pH of milk after the addition of sodium chloride. A reduction in pH was also obtained when sodium chloride was added to solutions of sodium citrate or phosphate. With the serum of neutralized cream, butter or milk, where an alkaline solid phase (e.g. calcium phosphate or citrate) is present, the change in pH was either diminished or reversed. Other factors are listed which were found to cause pH differences between salted butter and the cream from which it was made. White (370) has pointed out that in the neutralization of cream for the production of butter, the initial cream acidity, the weight of cream and the amount of neutralizer required must be determined accurately if butter of the desired pH is to be obtained. McDowall (371) has provided a table relating the neutralizing value of sodium bicarbonate to the acidity of cream in which allowance is made for the varying concentration of dissolved carbon dioxide at different levels of acidity. Bird (372) has found cream neutralized by the addition of sodium sesquicarbonate at 60° F. and agitated for 15 min. to give better butter, but slightly greater fat losses in the buttermilk, than cream neutralized by the rapid addition of the sesquicarbonate at 140° F. Adriani & Tamsma (373) obtained the best butter when cream ripening, churning and butter washing were all carried out at 14° C. Sandelin (374) has developed a microscopic technique for the estimation of fat losses in churning. A method for the microscopic examination of butter, which has been found of particular value in determining the quality of butter manufactured by the Alfa process, has been described in detail by Mohr & Eysank (375). Fritz (376) has studied the different physical conditions produced by different manufacturing processes in ordinary, Fritz and Alfa butter. Ordinary butter and Fritz butter had the high free fat content of 48 and 62% respectively, whereas Alfa butter had a free fat content of only about 10%. Alfa butter showed converse physical properties to those of ordinary or Fritz butter (e.g. a tendency towards oiliness as opposed to greasiness or crumbliness), mainly because the free fat content in Alfa butter contained only the fractions of low melting-point. A suitable procedure for the neutralization of ghee of high acidity is described (377).

Linneboe (378) has shown that the iron content of finished butter depends on the iron content of the water used for washing. A flavourless and highly stable carotene concentrate suitable for the colouring of butter has been prepared by Richardson & Long (379). Its effect on the develop-

ment of oxidized flavour is determined by storage conditions and the copper content of the butter.

Spaght (380) has given an account of the German process of manufacturing a synthetic butter from Fischer-Tropsch paraffin wax.

Hostettler (381) has discussed the role of 'free' and 'bound' water in cheese and other foods, and has pointed out that there is an optimum moisture content for hard cheese, which varies inversely with the fat content. Attempts to increase cheese yield by raising the moisture content above the optimum level affected the keeping quality adversely.

V. Defects of milk and milk products

(*a*) *Adulteration and its detection*

Added water

The freezing-point test. A valuable report on the Hortvet test has been presented by a Scottish Committee (382). The subject has been reviewed by Macdonald (383). Aschaffenburg & Temple (384) determined the freezing-point and solids-not-fat content of the milk of three Shorthorn cows throughout a lactation period. The variations in freezing-point were small, the extreme values being −0·529 and −0·560° C. Data for the freezing-point of morning and evening milk of individual Shorthorn cows sampled at short regular intervals for well over a year have been collected by Aschaffenburg & Veinoglou (385). The general mean was −0·548° C. Small seasonal variations were observed, and unusually high freezing-points were found after cows had been grazing on lush spring pasture. Systematic differences between the freezing-points of morning and evening milk were bound up with environmental temperatures. The application of the freezing-point test in detecting added water is discussed. In a third paper, Aschaffenburg, Temple & Veinoglou (386) report the results of freezing-point surveys of producers' milk as delivered to creameries in England and Wales. About 6% of the samples were suspected to contain extraneous water. Severe adulteration was rare. The variations in the freezing-point of the evening milk of twenty-eight cows over a 4-day period have been discussed by Tankard & Bagnall (387). Lighthelm (388), in South Africa, obtained a mean freezing-point of −0·541° C. (183 samples), the values ranging from −0·524 to 0·555° C. Authentic cases of genuine milks with exceptionally high freezing-points are rare. To the short list, Koenig (389) has added observations on a herd of four cows which were in a state of semi-starvation. Freezing-points as high as −0·480° C. were recorded, and were accompanied by unusually high blood values. On improving the diet, the freezing-points returned to normal levels after being exceptionally low (0·581–0·591° C.) for a short time. With virgin Shorthorn heifers in which lactation was induced by hormone treatment, Veinoglou (390) found slightly lower milk freezing-points than with control cows. Dodd & Cowan (391) observed normal freezing-points with mastitis-infected cows which gave milk of low solids-not-fat content. That mastitis, unless severely clinical, does not affect the milk freezing-point was shown by Rowland, Aschaffenburg & Veinoglou (392) in experiments based on inter-quarter comparisons. O'Loughlin & Ryan (393) recorded normal freezing-points in milks of low solids-not-fat content. Monk (394) has stressed the value of the freezing-point test in such cases. A method for the estimation of the original freezing-point of milk preserved with formalin has been described by Macdonald (395), and Ryan (396) has suggested a scheme by which the original freezing-point of very old samples of sour milk can be estimated from analytical data.

According to Kothavalla, Bhalla & Lavangia (397), there is little difference between the mean freezing-point and range of genuine buffalo's and cow's milk, and according to Gutierrez (398), the same applies to the Philippine carabao.

Other methods. Lythgoe (399) has described the procedures used by his laboratory to select, from thousands of milk samples, those suspected of adulteration, and the combination of special tests used to confirm suspicion. He does not consider it safe to rely solely on the freezing-point test. Seixas (400) has discussed the value of various methods for detecting added water. Gross (401) and de Vleeschauwer & Waeyenberge (402) have given further consideration to the use of methods based on determining the refraction of milk sera.

Rangappa (403) has shown that the refractive index of skim milk is identical with that of whole milk, and that its measurement can be used for detecting gross adulteration with water. According to Mokrants (404) low results for the titration of phosphoric acid in a trichloracetic acid serum indicate the presence of extraneous water. A combination of density and conductivity determinations forms the basis of a method recommended by Montefredine (405), whilst Occhialini (406) combines the results of determinations of the density of acetic acid serum with those of chloride determinations. Aschaffenburg (407) found Blegen & Rehberg's method for the determination of the osmotic pressure of biological fluids unsuitable for the detection of added water in milk.

Other types of adulteration

Rowlson & Mickle (408) recommend determination of the protein:fat ratio with milk samples suspected of fat abstraction, a ratio exceeding 1:1 being indicative of skimming. Moir (409) has discussed cases of 'adulteration' of milk with cream which have occured in New Zealand, where milk for cheese-making is paid for on a fat basis. Tests for the detection of reconstituted milk in normal milk have been described by King & Schouest (410) and in an Indian paper (411). According to Liebenthal & Adolph (412), soybean milk present in cows' milk can be detected with an accuracy of $\pm 2\%$ by a method based on nitrogen analysis of the precipitated glycinin. A test for the detection of additions of coconut 'milk' to milk of the cow or the Philippine carabao has been given by Gutierrez & Sunico-Suaco (413).

Various forms of adulteration of human milk have been discussed by Smith (414), and methods for its differentiation from that of other species by Zaribnicky (415). To distinguish human from cows' milk, Rodkey & Ball (416) have developed methods based on the marked differences in xanthine oxidase content.

A number of papers on methods for detecting adulteration of ghee (417–422) indicates that this problem still remains largely unsolved.

(*b*) *Flavour defects of milk and milk products*

Oxidized flavour has been the subject of numerous recent papers. Krienke (423) examined milk samples from all the cows in a herd regularly over two lactation periods. Many cows produced rancid milk either occasionally or for prolonged periods. No specific causes or factors were observed which would explain these anomalies. Corbett & Tracy (424) tested milk samples from individual cows over the period March to September for the development of spontaneous and copper-induced oxidized flavour. Early lactation favoured the development, and milk from heifers was more susceptible than that from older cows. Freeman (425) examined bulk milks from two sources regularly for one year and made the unusual observation that susceptibility was reduced during the months of November to February.

Josephson, Burgwald & Stoltz (426) studied the effect of exposure to sunlight on the ascorbic acid, riboflavin and flavour of milk; exposure for 30 min. in $\frac{1}{2}$-pint bottles resulted in a loss of 98·4% of reduced ascorbic acid, and in the development of off-flavours. Using special glass cells, Josephson (427) demonstrated that 'sunlight' flavour is due to light of wave-length 590–630 mμ, which is able to penetrate three glass partitions and 0·6 in. of milk. Danielsson (428) found that the effects of light were retarded, but not eliminated, by the use of milk cartons; brown glass bottles gave good protection. The effects of sunlight were not significantly altered by sizing ordinary paper containers with a layer of finely milled oat flour, as shown by England & Wiedemer (429). Trout, Bryan & Horwood (430) obtained no marked differences in flavour development when milk was cooled by varying methods.

Keeney & Josephson (431) observed that intense flavour developed in the protein-free serum of skim milk exposed to sunlight. No off-flavours occurred with skim milk which had been dialysed. When skim milk was dialysed after flavour development, no flavour could be detected in the dialysable fraction, and little in the non-dialysable.

The complex nature of the oxidation-reduction potential of milk and butter serum has been reviewed and investigated in detail by Saal & Heukelom (432), with particular regard to its relation

to oxidized flavour development. For milk, the tendency to develop off-flavours was well indicated by increasing E_h values, but for butter little useful information could be obtained by measurements of the potential. The effects of various metals on the E_h of milk have been studied by Hartman, Garrett & Button (433). Only copper, and, to a slight extent, divalent iron increased the E_h values.

Corbett & Tracy (434) found no correlation between the tendency of milks from individual cows to develop oxidized flavour and the iodine number of the milk fats. According to Horrall & Epple (435), oxidized flavour is unrelated to the natural copper content of milk. The same authors (436) found little variation in copper values (0·21–0·24 p.p.m.), the values being highest during early lactation. They discuss the effects of copper contamination and of ascorbic acid losses during the processing of milk. The role of ascorbic acid has been studied in detail by Krukovsky & Guthrie (437). Partial destruction through oxidation by light or hydrogen peroxide promoted the development of flavour defects. After complete destruction of ascorbic and dehydroascorbic acid, milk kept well during prolonged cold storage even if copper had been added. Later (438), the authors reported that flavour defects in cold-stored pasteurized milk developed most rapidly when the milk contained approximately equal amounts of ascorbic and dehydroascorbic acid. The effects of hydrogen peroxide were found to depend on its concentration. Guthrie (439) has shown that freedom from oxidized flavour can be achieved by passing milk through a de-aeration plant before pasteurization. There were only moderate losses in ascorbic acid when deaerated samples were stored for a week. Similar favourable results were obtained by Leeder & Herreid (440) on milks stored under a partial vacuum. Using the dropping mercury electrode, Hartman & Garrett (441) have studied the relationship between the oxygen dissolved in milk and the oxidation of ascorbic acid. Olson & Brown (442) found no development of copper-induced oxidized flavour in washed cream, unless ascorbic acid was added. Similar effects were obtained by adding hydrogen peroxide, suggesting that the peroxide liberated during the oxidation of ascorbic acid causes oxidized flavour by its interaction with the milk phospholipids. In further papers (443), Olson & Brown have dealt with inhibitors of oxidized flavour, which may be broadly classified into those that decrease the ionization of copper salts and those that are themselves oxidized by hydrogen peroxide, thereby protecting the phospholipids. The inhibitory action of certain amino-acids and of the albumin-globulin fraction of milk has been demonstrated by Hartmann (444). This author has shown that the fatty fraction of milk contains only a small percentage of the copper naturally present in milk. Castell (445) has observed that small additions of ammonia to milk may accelerate the development of oxidized flavours.

The addition of condensed or dried milk to liquid milk suppresses oxidized flavour, according to Russell & Dahle (446), whole-milk products being more effective than skim-milk products. Tarassuk & Henderson (447) have observed the presence of a powerful antioxidant in a number of commercial preparations of the vitamin B complex. The active principle which was alcohol-soluble could not be identified with crystalline members of the B-complex either singly or in combination. Later (448), the authors found tannins and flavones to be strong antioxidants. The efficacy of tea extracts was greater than that expected from their tannin content. Mueller & Mack (449, 450) observed antioxidative properties in crude and, to a lower degree, refined cane, beet and corn sugar. Cacao shell and cocoa were also active. The addition of 1 part of pancreatin to 70,000–140,000 parts of milk or cream has been patented by Stevens (451) for the prevention of oxidized flavour. Nelson (452) observed a retardation of flavour development when small amounts of pancreatic enzyme were added to raw milk just before pasteurization.

The relationship between the development of cooked flavour and the sulphydryl production in heated milk fractions has been studied by Patton & Josephson (453). According to Hankinson, Ellertson & Gleim (454), milk contains a dialysable cooked aroma precursor which differs from that originating from heat-coagulated proteins. Work on its isolation proved it to be rather elusive. Josephson & Keeney (455) have suggested that 'cowy' flavour in winter milk may be due to the transfer of acetone bodies from the blood of cows suffering from ketosis or acetonaemia.

The relationship of fat acidity to rancidity in homogenized raw milk has been studied by Gould (456). Moore & Trout (457) found that organoleptic examination of pasteurized homogenized

milk after holding for 24 hr. at 40° F. was not a sensitive test for contamination with raw milk; unless more than 4 % of raw milk had been added, rancidity could not be detected with certainty. A number of factors which cause and prevent oxidized flavour in frozen cream has been investigated by Barnhart (458), who found phospholipid changes of major importance in flavour development. Experiments by Stull, Herreid & Tracy (459) showed nordihydroguaiaretic acid to be an effective antioxidant for tinned frozen cream stored at −5 to −20° F. for periods up to 9 months.

The causes of oxidative deterioration of stored butter have formed the subject of several detailed studies. Two reports by van der Waarden (460, 461) deal, in particular, with the role of metals and with the nature of the substances which cause flavour defects. In unsalted butter made from sour cream, all of the iron and much of the copper was found to be bound to the protein. Most of the remaining copper was associated with the phospholipids. Contrary to these metals, manganese was not bound up with the colloids of butter. Attempts were made to isolate and characterize the substances responsible for flavour defects in cold-stored butter. Investigation of concentrates obtained by molecular distillation suggest that these substances are of an aldehydic nature, of low molecular weight, and free from nitrogen, sulphur and phosphorus. Using a Warburg technique, Hartmann (444) has demonstrated the copper-induced oxidation of lecithins, and has found that this type of reaction results in the development of oxidized flavours. In cold-stored samples of butter, made under controlled conditions and with addition of varying amounts of copper, the intensity of oxidized flavour showed a positive relationship to the copper content. Davies has published a series of papers (462) dealing with the deterioration of butter during storage. Various factors were studied which induce fishiness and subsequent tallowiness in stored butter. The value of a number of chemical tests in detecting oxidative deterioration was assessed. The titratable acidity in 96 % alcohol was found to increase markedly in tallowy, and drastically in mouldy butter. The free fatty acids of such butters consisted largely of oleic, palmitic and stearic acid. Oiliness, accompanied by fishy and, finally, rancid odour was induced by *Oospora* and *Oidium*, whilst *Penicillium*, *Monilia* and *Cladosporium* caused ketonic rancidity without oiliness. Krienke & Fouts (463) prepared butter from samples of cream 'contaminated' with various cultures of micro-organisms, and assessed the comparative value of organoleptic and chemical tests in detecting the defects developing on storage of such butters.

Hussong & Hammer (464) have described a number of cases of bleaching and tallowiness in commercial butter in which copper appeared responsible for the defects. Hussong, Quam & Hammer (465) have suggested that low pH and high copper content promote fishiness in butter. Bird & Fabricius (466) found butter to keep best when the serum had a pH of about 6·7, and Aas & Borgen (467) found the keeping quality the poorer the lower the pH of the butter serum. Jørgensen (468) has pointed out that butter colouring may be the cause of oxidative spoilage due to the development of peroxide during storage. Swartling (469) compared the keeping quality of ordinary butter and of Alfa butter, using samples with a pH of 4·7 in the serum. Flavour defects were much more pronounced with Alfa butter, the spoilage being ascribed to the larger interfacial area fat/water in this product.

Olson (470) has dealt with a butter defect occurring in Oklahoma, and known as 'skunkweed' flavour, which appears to be due to the ingestion of the western daisy (*Bellis integrifolia*) by grazing cattle. New Zealand workers have published a series of reports (471, 472) on land-cress taint. 'Winter' flavour, due to lipase action, has been discussed by Hansen & Theophilus (473) and by White & Gibson (474).

Maleic acid has been listed as one of a series of powerful fat antioxidants by Sabalitschka (475), but Glimm & Nowack (476) and Kaufmann & Wolf (477) have reported unfavourably on this compound. Good antioxidative properties were observed by Gould (478) for Avenex concentrate, a water-soluble cereal extract. Davies (479) has demonstrated that deterioration may be markedly delayed by wrapping butter in parchment paper coated with a cereal flour paste.

El-Rafey, Richardson & Henderson (480) have studied the effect of the method of manufacture of butter oil on its keeping quality, and have obtained the best results with the boiling-off process which involves heating to high temperatures (up to 130° C.). To prevent oxidative spoilage of dry butterfat, Holm, Schaffer & Haller (481) have recommended reduction of the dissolved oxygen to

less than 0·5 volume %. Glimm & Ellert (482) found drying of butterfat with silica gel effective in retarding acidity development. Dried samples, stored in the dark, did not form peroxide. According to Solntsev (483), the presence of casein in dry butterfat accelerates deterioration when samples are stored under exposure to light and air. Lea (484) has studied the antioxidative value of numerous organic compounds added to butterfat. Good results were obtained with ethyl gallate in low concentration. The importance of protection of butterfat against light has been discussed by Hinko (485). The need for elimination of the wave-lengths that are absorbed by the carotenoids is stressed, as only these are active in the bleaching process.

Australian workers have published papers (486, 487) dealing with factors affecting the keeping quality of 'concentrated hardened butter', a product prepared by the addition of hydrogenated peanut oil to butterfat. Achaya (488) has analysed samples of ghee stored for several years at 15–25° C. The results showed marked oxidation of unsaturated fatty acids with production of lower acids.

Dahle & Josephson (489) prepared three types of dried milk with varying lecithin content. On storage, with or without nitrogen packing, the samples with the lowest lecithin content had the best keeping quality.

Schaffer, Greenbank & Holm (490) have studied the development of tallowiness in milk fat and dried milk stored under gas mixtures of varying oxygen content, and have demonstrated the importance of low oxygen concentration. Air-packed samples of spray-dried whole milk were found by Coulter & Jenness (491) to deteriorate at a reduced rate when the ascorbic acid had been oxidized by hydrogen peroxide before preheating. The great value of gas-packing has been stressed by Remaley (492), who has also assessed the usefulness of a number of antioxidants. Williamson (493) incorporated animal and vegetable fats into dried skim milk, and tested the effect of a variety of added compounds on the oxidation of the fats. The efficacy of several antioxidants has been evaluated by Hollender & Tracy (494). The possibilities of improving the keeping quality of milk powders by the addition of wheat-germ oil have been thoroughly investigated by Tracy & Hoskisson (495) and by Chapman & McFarlane (496); the results were not encouraging. Out of a series of antioxidants added to spray-dried whole-milk powder, ethyl gallate gave the most favourable results in experiments by Findlay, Smith & Lea (497).

Factors promoting and abating lipolytic flavour defects in Cheddar cheese have been studied by Hlynka, Hood & Gibson (498, 499, 501) and Hlynka & Hood (500).

VI. Analysis of milk and dairy products

(*a*) *Milk*

Fat

Chillingworth & Gillespie (502) found 'direct' sampling less reliable than 'drip' sampling in assessing the fat content of milk contained in a shallow weigh-vat. Whitman, Slack & Herreid (503) found the amount of milk fat delivered by 113 creamery suppliers to be lower by an average of only 1 in 200 when estimated from monthly instead of the usual fortnightly composite tests. Close agreement between monthly average fat contents based on daily testing and on testing three or four times a month was obtained by Hellstrand (504).

Andersen (505) has found a syringe of the ordinary medical type to compare favourably in speed and accuracy with an 11 ml. pipette when used for measuring milk for fat determinations.

Existing knowledge of the influence of phosphatide on the determination of fat in milk and milk products has been reviewed by Schloemer, Rauch & Schloemer (506).

Literature on the Babcock test has been reviewed by Herreid (507). Herrington (508) has calculated speeds for Babcock centrifuges between 10 and 24 in. diameter which yield equal forces at points 1½ in. above the bottom of the cups (i.e. at the fat-acid interface during the first centrifuging period). He considers that with the smaller machines (10–14 in. diameter) the centrifugal force at the bottle necks is insufficient to remove all adherent water.

The average density of pure butterfat, substantially independent of source, has been reported

by Jenness, Herreid, Caulfield, Burgwald, Jack & Tuckey (509) and Martin, Caulfield, Beck, Nelson, Foltz, Whitnah & Parsons (510) as 0·8892 and 0·8896 g./ml. respectively at 140° F., and by Lampert (511) as 0·8925 g./ml. at 135° F. Fat extracted in the Babcock test gave a slightly higher, and more variable, value (509, 510, 511) due to the presence of a varying quantity of non-fatty material (511, 512). All values were below 0·9 at 140° F., the value assumed in the calibration of Babcock tubes. Jenness & Herreid (513) point out that, in practice, this is approximately compensated for because the fat column is usually below 140° F. at the time of reading.

With the Mojonnier technique, Herreid & Harmon (514) prefer to measure samples by volume or to weigh them direct into the extraction flask to the weighing of filled pipettes in which fat may rise during weighing and adhere to the sides. Herrington (515) noted a change of about 0·75 mg./°C. when Mojonnier fat dishes were weighed directly from cooling ovens that were not at atmospheric temperature. The error introduced may be eliminated by replacing the normal water-cooling system by an air-cooled radiator, which follows room-temperature changes more rapidly.

Figures illustrating the reproducibility of the Mojonnier method have been provided by Herreid & Whitman (516). The Babcock method has been shown (516, 517) to give a fat content slightly higher than the Mojonnier technique. The two methods have been compared in accounting for milk fat on a laboratory (518) and factory scale (519). Fill & Stock (520) have described an adaptor for the acid pipette used in the Gerber test, which prevents contamination of the neck. Cador & Macherel (521) have further studied the errors involved in the graduation of Gerber butyrometers, and suggest that details of the basis of graduation should be supplied with each butyrometer. A method for the estimation of fat in milk using a special alkaline reagent and no centrifuge (522) has given results in agreement with Gerber tests.

Janse (523) has compared the Röse-Gottlieb, Weibull and Gerber methods. The Weibull method was preferred as being more accurate than the Röse-Gottlieb, and is described very fully. Boiling with a mixture of hydrochloric acid and carbon tetrachloride to disperse the non-fatty constituents prior to the quantitative extraction of fat in dairy products and other foods, is recommended by Schloemer & Rauch (524). The low boiling-point of this mixture minimizes the formation of non-fatty decomposition products soluble in fat solvent.

Reid (525) has detailed special precautions which must be observed in applying the Gerber test to sterilized milk.

The unsatisfactory nature of the various rapid methods of estimating the fat content of homogenized milk has been noted by Trout & Lucas (526) and others (527, 528). Trout & Lucas (529) have further considered various modifications of the Babcock procedure suggested for homogenized milk, the best features of which were combined in a new technique (530). The main points are adjustment of the milk and acid to 70° F., addition of 17·5 ml. of conc. sulphuric acid in three portions, with intermediate and a final prolonged agitation, and the addition of a mixture of water and alcohol (1·4:1), instead of water alone, before centrifuging. The mean results for thirty-six samples of milk before and after homogenization were 0·058 and 0·045 respectively, higher than those obtained by the Mojonnier method. A similar method has been described by Marquardt (531).

Hansen & Deane (532) have compared various American rapid methods for the estimation of fat in skim milk with the Mojonnier test. In a later paper (533), the centrifugal methods most suitable for skim milk, butter-milk and the whey from various cheeses have been detailed. Hostettler, Lehmann & Künzle (534) have similarly compared several butyrometer methods and various modifications of the Röse-Gottlieb technique.

Mohr & Baur (535) have described improved fat tests for butter serum and sweet-cream buttermilk. However, Sandelin (536) has observed that their modification for buttermilk does not prevent the formation of a solid layer below the fat column. Mulder (537) has found that formalin added to prevent the formation of this layer tends to reduce the volume of the fat column.

Dry extraction with chloroform has been recommended (538) for the determination of fat in cream and ice cream. An indirect method for the estimation of fat in butter was applied to cream by Mohr & Pasveer (539) and gave results comparing favourably with the Röse-Gottlieb and Weibull

methods. Schwarz & Mumm (540), Heinemann (541) and Hurel & Mocquot (542) have also given attention to methods for the estimation of fat in cream.

Sandelin (543) has estimated the efficiency of milk separation by a microscopic counting of the fat globules in the skim milk. Mulder (544) considers microscopic counts of fat globules exceeding 2 μ in diameter suitable for judging the performance of separators. He points out that this type of technique avoids the errors caused by the fat-like material when solvent extraction methods are applied to skim milk.

Mulder (545) has described a modified Golding method suitable for the isolation of butterfat from milk and cream when a determination of refractive index is required. A method for the estimation of fat crystallization in cream has been worked out by Adriani & Tamsma (546). Variations in the acid degree of milk fat introduced by churning and extraction procedures have been studied by Johnson & Gould (547).

Total solids

The Society of Public Analysts' method for determining milk solids has been detailed (548). A 5 g. sample of milk, weighed into a prescribed metal dish, is dried for 30 min. on a boiling water-bath and then for 3 hr. in a well-ventilated oven at 98–100° C. Heating for periods of 1 hr. and reweighing are continued until the further loss in weight does not exceed 0·5 mg. Livak (549) and Doan & Livak (550) have described the estimation of total solids in whole and processed milks and in sweetened-condensed milk and ice-cream mix by the Dietert Moisture Teller method, in which the samples are dried by forced heated air. The deviation of results from those obtained by the official A.O.A.C. and the Mojonnier method was small.

Stuart & Martin (551) have described an improved Westphal balance in which a chainomatic principle takes the place of riders.

Using the method of British Standard Specification no. 734, 1937, Bakalor (552) found average densities of 1·0288 and 1·0285 at 20° C. for two series of milks. Slight seasonal fluctuations were noted. Bohm (553) has published a simple table for the calculation of solids-not-fat contents from fat and specific gravity presumably based on the Fleischmann formula. Boden & Campbell (554) found solids-not-fat percentage calculated by Richmond's formula to agree with values given by their evaporation technique only when the specific gravity was determined on samples exhibiting complete Recknagel contraction. When results were calculated by the Richmond specific gravity formula and by the British Standard Specification density formula derived therefrom, for samples with no Recknagel contraction they were 0·2 % lower than their evaporation results. The authors emphasized the need for a standardized evaporation technique as a preliminary to a revision of the B.S.S. formula. Desai & Patel (555), in a comparison of Richmond's formula and a gravimetric procedure, found increased discrepancy between the two figures for total solids when water was added or fat abstracted.

In the Sharp & Hart equation [*J. Dairy Sci.* **19**, 683 (1936)] for calculating milk solids, the specific gravity is expressed on the 86° F./86° F. basis. Rueda (556) has given the appropriate corrections for adjusting to this basis lactometer readings based on water at 60° F. Herrington (557) has also modified this equation for the same purpose. Livak (558) used two suggested corrections for adjusting values from the 86° F./60° F. basis to the 86° F./86° F. basis. The mean total solids figures calculated were 0·228 and 0·301 % higher than those obtained by the Mojonnier technique.

Schnecke & Plottke (559) have provided formulae for the calculation of total solids of the milks of Karakul and Merino sheep from their specific gravity and fat content. Refractive indices ranging from 1·3474 to 1·3506 for cows' milk and from 1·3484 to 1·3534 for buffalo's milk have been obtained by Rangappa (560). It was found that the major milk constituents make differing contributions to the total refractive index. Consequently there is no exact linear relationship between refractive index and solids-not-fat.

Nitrogen and its fractions

Chibnall *et al.* (101) consider that in many laboratories too short a digestion period is used in the Kjeldahl procedure. After the digest from proteins or protein hydrolysates had cleared, heating

for a further 8 hr. or more was found necessary to give complete recovery of nitrogen. From data presented by Alcock (561), it appears that the digestion period used by Chibnall *et al.* might be shortened without loss in accuracy if the concentration of potassium sulphate were increased. Alcock himself has recommended a *total* digestion period of 6 hr.

Sarudi (562) has described a rapid Kjeldahl procedure in which 0·5–1 g. of dry matter is digested with 3–5 ml. of sulphuric acid and 13–15 ml. of 30% H_2O_2 for 15–20 min. Accuracy is unimpaired by the reduction in digestion time. Jonnard (563) has developed a reliable semi-micro technique, using hydriodic acid and selenium oxide with a digestion period of 12–16 hr., for total nitrogen estimations on biological fluids containing precipitants of the tungstic acid type. Miller & Houghton (564) consider that for accurate nitrogen determinations on β-lactoglobulin by the micro-Kjeldahl method, the copper catalyst must be replaced by mercuric oxide. The method of Levy & Palmer [*J. biol. Chem.* **36**, 51 (1940)] for the estimation of ammonia by the addition of sodium hypobromite in excess and back-titration with thiosulphate has been applied by Ling (565) to micro-Kjeldahl digests of milk, milk filtrates and whey. The method, which avoids the lengthy distillation procedure, was found sufficiently accurate for most purposes.

Münchberg & Sulinskas (566) have determined non-protein nitrogen in milk by a conversion to ammonia which is then estimated colorimetrically with Nessler's reagent. Choi, O'Malley & Fairbanks (567) have utilized the colour reaction of ammonia with alkaline phenol and sodium hypochlorite for its estimation in milk and dry-milk products.

Thomé (568) has determined average factors for converting the results of various forms of the formol titration into milk casein, using the method of Schlossmann (6·45 × Kjeldahl N of alum precipitate) as the standard. In a later paper (569), he has described optimum conditions for the formol titration and considers it sufficiently accurate for the adjustment of the fat content of cheese milk. Overby & Madsen (570) have compared the estimation of protein by formol titration and by calculation from Fleischmann's formula with the Kjeldahl method. Brereton & Sharp (571) have given details of an improvement in Robertson's refractometric method for the estimation of casein in skim milk. When estimating casein by the biuret colour reaction, Wokes & Still (572) found the results to increase with the ageing of the samples. Doherty & Ogg (573) have modified the standard manometric Van Slyke apparatus for routine determinations of the free amino-nitrogen content of solid proteins, including casein, and protein derivatives. Fraenkel-Conrat & Cooper (574) have developed a micro-method for the estimation of β-lactoglobulin, based on its precipitation with the acid dye 'orange G' and the colorimetric measurement of the dye remaining in solution.

Virtanen & Rautanen (575) made a quantitative micro-determination of the combined total of alanine, valine, leucine, *iso*-leucine, phenylalanine and methionine in casein by estimating in bisulphite solution the aldehydes distilled from a hydrolysate treated with ninhydrin. These amino-acids accounted for 24·6% of the protein nitrogen. Hauschildt, Isaacs & Wallace (576) found the method of Eckert [*J. biol. Chem.* **148**, 205 (1943)] to give reproducible results and a good recovery when applied to the estimation of tryptophane in casein.

An analytical procedure for the separation of the nitrogenous fractions of skim milk has been developed by Harland & Ashworth (130). Casein is removed by saturating the skim milk with sodium chloride at 40° C., standing overnight and filtering with a filter aid. The soluble proteins are precipitated by bringing this filtrate to pH 2 by the addition of hydrochloric acid. In a comparison with the generally accepted method of Rowland, slightly lower soluble protein nitrogen and slightly higher casein nitrogen, but similar non-protein nitrogen values were obtained. Using phosphate concentrations of 1·35, 1·54 and 1·71 M of an equimolecular mixture of KH_2PO_4 and K_2HPO_4 as protein precipitants, de Vleeschauwer & Heyndrickx (577) have separated the proteins of milk into four fractions. The first three fractions account approximately for the casein nitrogen, while the fourth fraction corresponds approximately to the combined proteose, globulin and albumin fractions.

Horvath (578) has reviewed the value of metaphosphoric acid as a deproteinizing agent. The advantages of using perchloric acid instead of trichloracetic acid for deproteinization have been pointed out by Neuberg, Strauss & Lipkin (579).

Minerals

Pyne(580) has suitably adapted the macro-procedure of Washburn & Shear [*J. biol. Chem.* **99**, 21 (1932)] to the micro-determination of calcium and magnesium in a trichloracetic acid-milk serum. Wuhrmann & Högl(581) have applied to milk and cheese the colorimetric method for the estimation of phosphorus in foodstuffs of Fiske & Subbarow as modified by Teorell [*Biochem. Z.* **230**, 1 (1931)]. Removal of organic matter by either wet or dry ashing, followed by the above method, gave results agreeing closely with those of a gravimetric procedure. Micro-sedimentation techniques for the estimation of phosphorus and potassium in milk have been developed by Vladesco(582, 583). Sendroy's iodimetric chloride method has been applied to protein-containing fluids, including milk, by Van Slyke & Hiller(584). With the necessary precautions, the percentage error does not exceed 0·2. Grossfeld & Young Yen(585) have estimated the sulphate in milk by precipitation of the barium salt from an acetic acid-potassium ferrocyanide-zinc acetate serum. For thirty-five samples, a mean sulphur trioxide content of 7·96 mg.% (range 6·83–9·08) was obtained.

Methods for the estimation of copper and iron in cream, which are similar to the wet-ashing and simplified filtration procedures used for butter (see § VI (*b*)), have been described by McDowell(586). Wet-digestion and filtration procedures for copper determinations in cream have also been given by DeAth, Lightfoot & Moir(587). Hartmann(444) has developed an electrolytic-spectrographic method of determining copper in milk and butter which is ten times as sensitive as the dithizone method. Cranston & Thompson(588) used an ion-exchange resin for the separation of copper, later estimated polarographically, from a perchloric acid-milk serum. Woiwod(589) obtained a good recovery of iron from milk by precipitating it as ferrous sulphide from the ash solution and then estimating it colorimetrically as the $\alpha\alpha'$-dipyridyl. The formation by cobalt of a coloured *o*-nitrocresol complex, which may be concentrated by extraction into ligroin, has been used by Ellis & Thompson(590) in the determination of the small quantities of cobalt in milk. Owen(591) has described a method for the micro-determination of boron, based on the ability of boron to deepen the bluish purple colour developed by quinalizarin with concentrated sulphuric acid. The boron is isolated from an alkaline ash by distillation as methyl borate. A rapid test for borate which may be applied to milk direct has been evolved by Bonoldi(592). The 'Analytical Methods Committee' of the S.P.A. has published a standard method for the determination of fluorine in foods(593). After ashing the sample with lime, the fluorine is separated as hydrofluorosilicic acid by distillation in the presence of excess perchloric acid, and then estimated by a titration depending on the bleaching action of fluorine on a thorium alizarin sulphur lake. A value of 0·17 p.p.m. is reported for a sample of milk.

For the rapid detection of neutralizer in milk, Mühlschlegel(594) utilized the observation that the reduction of the titratable acidity of milk which occurs on boiling is increased when added carbonates are present.

Miscellaneous

Garrison & Haigh(595) have continued their study of the variation in the volume of the protein-fat precipitate and its effect on the accuracy of the polarimetric determination of lactose in milk. Rauschning(596) describes a method for lactose in which the copper sulphate remaining after a Fehling reduction is estimated colorimetrically as a copper-ammonium complex. Peltzer(597) gives details of a colorimetric procedure which he has found suitable for estimating quantities of from 0·05 to 1·0% of added sucrose in milk. Heinemann(598) has adapted the cerate oxidimetric procedure of Smith & Duke [*Industr. Engng Chem.* (Anal. ed.), **15**, 120 (1943)] to the estimation of citric acid in milk and milk products. This titration method is much more rapid than the usual pentabromoacetone procedures, and gives results which are slightly higher. Eilers & Jense(599) have described a modification of the pentabromoacetone determination of citric acid which gives more reliable results than existing versions.

Zilliox, Mitchell & Frary(600) have suggested changes in the normal procedure for titrating milk which give lower apparent acidities than does the official A.O.A.C. technique. Hillig(601) has modified his colorimetric method for the estimation of lactic acid by using barium lactate

instead of lithium lactate for the preparation of standard solutions. A collaborative study (602) has shown the method to be generally satisfactory when applied to mixtures of lactic acid with formic, acetic and butyric acids. Gould (603) has confirmed the reliability of the method in general and in the presence of added preservatives, sodium chloride, sodium citrate, disodium phosphate, sucrose and developed rancidity, but on the addition of sodium carbonate, bicarbonate, sodium benzoate or potassium dichromate, values differing from the theoretical were obtained. While heat treatment increased the titratable acidity, it had little effect on the lactic acid figure (604). A general relationship between the increases in lactic acid and titratable acidity has been demonstrated by Gould & Jensen (605).

A colorimetric procedure for the estimation of acetylmethylcarbinol and diacetyl in starter cultures and sour cream, which has given results in good agreement with the gravimetric method of van Niel, is described by Zarins & Brants (606). Grossfeld & Zeisset (607) have observed that a more complete extraction of phosphatide from dairy products is possible with an ethyl alcohol-benzene mixture than with an *iso*-propyl alcohol-benzene mixture.

Townley & Gould (608) have described a method providing an arbitrary value for the heat volatile sulphides of milk. It is shown that an increase in the time or temperature of heating increases the volatile sulphide values. However, heating milk at 90–95° C. for long periods or in a sealed vessel, or heating for short periods at temperatures above boiling, decreases or prevents sulphide liberation. Diemair, Strohecker & Keller (609) have proposed a method for the estimation of the volatile substances in milk based on the reduction of hypochlorite. Hartman & Garrett (610) have successfully applied the dropping mercury electrode method to the measurement of the concentration of dissolved oxygen in milk.

Horn (611) has compared several tests for formaldehyde in milk. Methods for the estimation in milk of D.D.T. and quaternary ammonium compounds are reported in (612–614). Procedures for the estimation of acetone in milk and glycerol in whey are described (615,616).

Pedersen (617) has studied the relative merits of paraformaldehyde and bichromate tablets for preserving composite samples of milk. Pien (618) prefers hydrogen peroxide to bichromate for the preservation of samples for acidity tests.

A new procedure for the gravimetric estimation of the fat and solids-not-fat content of sour milk and for the estimation of the necessary corrections for decomposition products has been developed by Macdonald (619). The fat and solids-not-fat are separated from water and decomposition products by entrainment distillation of the sour milk with *n*-heptane. The procedure, which gave results in reasonable agreement with those obtained by testing the original fresh milks, appears to be a definite improvement, particularly as regards ease and speed of manipulation, over the established maceration procedure. The author also describes a rapid routine method based on Gerber tests and lactometry which gives results almost as reliable as does the gravimetric procedure.

The testing of dairy products for the determination of filth and extraneous matter has been discussed by Clarke (620). Rudnick (621) has adapted the 'off-bottom' sediment test for use with cream and has suggested its adoption as a standard procedure.

(*b*) *Butter*

Mennicke (622) has determined the air content of butter by centrifuging the melted sample in a specially designed tube with a narrow graduated end in which the volume of air is measured directly. By taking mean values for the percentages of water and solids-not-fat, and for the specific gravities of solids-not-fat and fat, he (623) has arrived at a formula by which air may be calculated from the specific gravity of butter. Mohr & Eysank (624) have also described a rapid and simple estimation of air in butterfat. Loftus-Hills & Conochie (625) have modified the method of Rahn & Mohr [*Milchw. Forsch.* **1**, 213 (1924)] for estimating the low gas content of 'concentrated butter'. A method for the oxygen content of butterfat described by Morell, Conochie & Loftus-Hills (626) involves the initial displacement of the air from the melted fat with nitrogen.

An iodometric procedure for the estimation of active oxygen in butterfat has been described by Sabalitschka (627). Parmelee, Bird & Breazeale (628), in a paper which contains many useful details

concerning technique and procedure, have compared the pH values of neutralized cream, and of butter made from this type of cream, using hydrogen, glass and quinhydrone electrodes and also a colorimetric method. They have investigated the effect of various factors on the differences between pH values recorded by different electrode systems. White (629) has found indicator paper unsuitable for the determination of the pH of butter serum, buttermilk and cream.

Vorhes (630) has reported on the proposed revision of the A.O.A.C. method for the preparation of butter samples. The sample is brought to a stage of 'optimum fluidity' before it is thoroughly mixed. With this preparation the moisture content is slightly higher and presumably more accurate than with more rapid preparations, such as that described by Meuron (631). Ritter (632) and Götel & Effern (633) have dealt with the errors that a bad sampling technique may introduce into moisture determinations. Mohr & Baur (634) compared several methods for estimating moisture in butter. With methods involving evaporation, results with rancid samples were less consistent than with fresh butters owing to the loss of volatile fatty acids. Götel & Effern (633) have suggested a modification of the rapid method suitable for serial determinations. Olsson (635) has replaced the open flame of the rapid method by a hot-plate, which obviates soot formation, lessens the danger of overheating and practically eliminates spurting. The accuracy of this modification has been confirmed by Andersen (636). When using Fischer's reagent for the rapid determination of moisture in butter and other dairy products, Heinemann (637) obtained values in agreement with the accepted methods; but the reagent required frequent standardization and vitamin C caused interference. Thiel (638) has defined several heating conditions which give moisture contents on dried butterfat in agreement with the direct Fischer method. Entrainment with nitrogen followed by adsorption on silica gel gave similar results to the Fischer method, whilst toluene distillation gave results 0·015% lower.

Korpáczy & Ersek (639) have evolved a composite method for the estimation of fat, water curd, salt content and acidity in butter. The sample is held in a Jena sintered crucible suspended over a crystallizing dish and, after removal of moisture by heating, the solids are freed from fat by washing with carbon tetrachloride. Salt is dissolved in water and titrated with silver nitrate. Acidity is determined on the extracted fat. Nussbaumer & Ritter (640) have used this approach for routine solids-not-fat estimations. Mohr & Mack (641) prefer to estimate fat in butter by difference, rather than by direct methods. King (642) has studied the effect of working butter on the percentage of globular fat, by microscopically counting and measuring the globules before and after the treatment.

Determination of the melting-point of butterfat by microscopic methods has been recommended by Mohr & Eysank (643), who consider it to be quicker and more exact than macroscopic procedures, and by Kaufmann & Lund (644) because it has the advantage that the melting-point of each constituent, or phase, may be noted. A 'softening point' has been proposed by Barnicoat (645) in preference to the melting-point determination, and he has devised a simple and reproducible method. The softening point is of particular advantage with fats of a wide melting-point range. A microscopic method for the determination of the refractive index of fats has been described by Kaufmann & Lund (644).

Kruisheer, den Herder, Krol & van Ginkel (646) have found simple approximate relationships, different for summer and winter, between Reichert-Meissl values, and iodine values or refractivity results, and have provided provisional equations, for use in summer and winter respectively, by which the firmness of butterfat may be estimated from either iodine or butyro-refractometer values. In (647) it is shown how an approximate estimate of the hardness of butter can be derived from a combination of the iodine and Reichert-Meissl values of the butterfat. Brouwer (648) has studied the relationship between the crystallizing tendency of butterfat and its chemical constants in connexion with the firmness of the butter.

Grant & White (649) have found a high negative correlation between flavour score and the fluorescence of a clear butter serum diluted with sodium acetate solution. Effern (650) attempted to relate flavour score with several chemical tests, but only the acidity of fat gave promising results. Kosikowsky, Dahlberg, Guthrie & Knaysi (651) found high flavour score to be associated in general with low butterfat acidity, but individual variations excluded even a rough classification of butter.

Schloemer (652) obtained only low correlation between grading value and fat acidity. The peroxide and aldehyde values of a large number of butter samples examined by Støren & Dovle (653) were not closely related to flavour score.

Knaysi (654) used several basic dyes for measuring the hydrolysis of fat. The base of neutral red was most suitable for general purposes, and gave results agreeing closely with titration using sodium hydroxide (655). The method has been found useful in butter grading (656). Rieman (657) has developed an accurate double-indicator method for the saponification number of fats and oils, which has advantages in time, reagents and apparatus over the standard method. Bird (658) has compared the Hanus, Wijs, Kaufmann and Rosenmund-Kuhnhenn methods for determining the degree of unsaturation of small quantities of fat. The Rosenmund-Kuhnhenn method gave results agreeing most closely with the iodine number determined by hydrogenation and was least affected by strength of reagent and size of sample. Mulder (659) has shown that the thiocyanogen number cannot be a better indirect measure of the hardness of butter than the iodine number because the two are highly correlated. Platon & Olsson (660) and Aas (661) have obtained high correlation between butyro-refractometer values and iodine numbers. However, Janse (662) warns against the indiscriminate use of such regression equations, since he finds them to vary, even for the same season, from year to year.

Lea (663) has improved the iodometric method of determining the peroxide value of fats and oils. Mummery (664) has introduced certain modifications into the Schibsted fat aldehyde test; while Mangold (665) has described a modification of the method of Schmalfuss [*Vorratspfl. u. Lebensmittelforsch.* **1**, 98 (1938)] providing reproducible and accurate results for the free aldehyde in deteriorated butterfat. Sundberg & Hultberg (666) have compared methods for the estimation of peroxides, aldehydes and methylketones in fats. Application of the methods to butterfats kept under different conditions showed the value of each test as an index of deterioration. An apparatus automatically recording decreases in volume when samples of fats are agitated in air or oxygen at 100° C. in closed vessels, has been described by Sylvester, Lampitt & Ainsworth (667). Usually the rate of absorption increased after an induction period. The curves obtained were similar to those for peroxide values (modified Lea method). Beier (668) has developed a chemical test for deterioration of fats which is based on the reduction of bichromate by volatile substances released from fat at 60° C. Kiermeier (669) studied various chemical methods for determining deterioration in butter and concluded that none of them was adequate to serve as an index of keeping quality.

Brouwer (670, 671) considers that simple linear equations relating fat characteristics such as the Reichert-Meissl and saponification value are too unreliable to be of use in the absolute identification of fats, and shows mathematically that derived equations of a higher order might be more useful. Schloemer (672) found the butyric acid number, increasingly used for the detection of fat adulteration, to be highly correlated to the Reichert-Meissl number. Details are given by Voorst (673) of a combination of fat constants by which cow's, sheep's and goat's butterfat may be distinguished.

Henderson & Jack (674) have found 'Skelly Solve *A*', a cheap low-boiling petroleum naphtha, a suitable solvent for a simple low-temperature fractionation of milk fat. Hamdi & Zollikofer (675) obtained a coarse fractionation of a liquid-fat column by fractional crystallization at successively lower temperatures. Gould & Johnson (676) have tried various procedures for the concentration of the free fatty acids of milk fat. Gould (75) has made preliminary studies of the changes in fat constants introduced by various extraction procedures, with a view to investigating the acids involved in lipolysis. A simple process for the separation of non-saponifiable matter from large quantities of fat has been described by Grossfeld (677).

Ramsey & Patterson (678) have given details of the separation and identification of the volatile saturated fatty acids (C_1 to C_4). After separation from non-volatile substances by steam distillation, they are taken up in chloroform containing 1% of *n*-butanol and are separated chromatographically on a column of self-indicating silica gel. Chloroform-butanol mixtures are used to develop the column. The acids are identified under the microscope as cerous formate and mercurous acetate, propionate and butyrate.

Bohm, Sturz & Jonas (679) and Arbuckle (680) have found the mercurimetric method (involving titration with mercuric nitrate, in the presence of ether, using diphenylcarbazone as indicator)

suitable for the rapid and accurate determination of salt in butter. The electrometric determination of chloride in butter is considered by Vilegzhanin (681) to give a truer indication of salt content than the silver nitrate method, which gave consistently high results on butters to which known amounts of salt were added.

Horrall & Crane (682) have reported further on the use of their adsorption column technique for the separation of the lecithin from butterfat extracted from milk and dairy products. A fractionation of the phosphatides was obtained by elution of the column with alcohol-benzene mixture. Treatment of the column with alcoholic potassium hydroxide caused considerable elution of the phosphatide fatty acids. Mulder (683) compared three methods for the estimation of phosphatide in buttermilk and other milk products. A phosphorus estimation on a fat extracted by the Röse-Gottlieb method and ashed with magnesium nitrate proved most suitable. Crane & Horrall (684) have studied Roman's method (precipitation of the periodide followed by titration with thiosulphate) for the estimation of choline in butterfat. After an adjustment of the conversion factor based on standardization against pure choline chloride, good recovery of choline added to butterfat was possible.

Full details of a wet-ashing technique for the preparation of filtrates in the estimation of copper and iron in butter, dry butterfat and cream by improved colorimetric methods have been given by McDowell (586). Simplified filtration procedures, which involve the rapid removal of protein by precipitation, are also described and are suitable for routine work, giving results in good agreement with the wet ashing method. Sirks (685) has examined several colorimetric methods for determining iron in butter wash-water. For the full recovery of iron by any of the methods, the initial removal or organic matter proved an essential preliminary.

Schwarz, Fischer & Hagemann (686) have described a method for the rapid estimation of manganese in butter by heating the ashed sample with a $K_2CO_3.Na_2CO_3$ mixture and comparing the colour of the molten mass with a series of standards. A more accurate, but lengthy, method is also given.

A report containing standard methods for the analysis of ghee has been published (687).

(*c*) *Cheese*

Mulder (688) has found the 'Wulf' pH tester satisfactory for the determination of the approximate pH of Edam cheese. Ritter (689) has described a narrow diameter tubular borer particularly useful in sampling young cheese when resealing is desirable.

A sampler and sampling procedure used in testing for extraneous material is described by Erekson (690). A standardized technique for extraneous matter in curd or cheese, using 10% sodium citrate as cheese solvent, is given by Hood & Sproule (691). The original citrate method of Spicer and Price [*J. Dairy Sci.* **21**, 1 (1938)] has been modified by Thibodeau (692) for use with small samples of cheese (*c.* 15 g.). Emulsification of the cheese with the citrate is accelerated by disintegration in a Waring Blendor. Turner, Rogers & Conquest (693) used boiling 1% orthophosphoric acid as a cheese solvent; rind was dissolved completely without visible digestion of extraneous material. Miersch & Price (694) have found the two solvents equally convenient. They emphasize the importance of microscopic examination of extraneous material to detect the source of contamination. Julien, Dumais & Thibodeau (695) have compared several methods of preserving cheese samples for extraneous matter tests. The introduction of 0·5 ml. of chloroform on a wad of cotton-wool into the sample bottle was most satisfactory.

Variations in the apparent moisture content of cheese due to differences in sampling technique have been reported by O'Shea & Lyons (696), who describe a rapid method for moisture, involving heating the cheese for 6 min. on a hot-plate at 180° C. A method proposed by Olsson (635) is essentially the same. A more lengthy 'rapid procedure', where moisture is removed by warming the cheese under a layer of oil containing salt, has been evolved by Gould (697).

For the determination of fat, Grossfeld & Zeisset (698) prefer extraction of the cheese with boiling hydrochloric acid, followed by carbon tetrachloride and petrol, to the usual Schmid-Bondzynski-Ratzlaff method.

Babel & Nelson (699) report that the Mojonnier method when compared with the A.O.A.C.

method gives lower values for the fat content of cheese, particularly when the fat has undergone extensive hydrolysis. The high results for fat content of cheese obtained by the van Gulik method, as compared with Gerber values, are attributed by Zeisset (700) to the formation of amyl esters and ether which dissolve in the separated fat.

A collaborative study, reported by Garard (701), has shown the fatty acid constitution of the fat extracted from cheese by different methods to vary with the pH of the solvent. The desirability of a satisfactory neutral extraction method is stressed. Smiley, Kosikowsky & Dahlberg (702) have provided a simplified extraction-distillation method for the determination of volatile fatty acids in cheese. A phosphoric acid-cheese mush is extracted with ether, from which the acids are removed by washing with alkali, thus eliminating the lengthy water extraction common to many earlier methods. The total volatile acids are isolated by separate distillation of the acidified alkaline extract, and of the residual cheese mush, in the presence of magnesium sulphate. This method has given results in good agreement with those obtained by the method of Hiscox *et al.* In (703) a more rapid, but less reliable, technique is described in which the volatile acids are distilled direct from a sulphuric acid cheese mush after the addition of magnesium sulphate, which raises the boiling-point of the mixture. In neither method is an elaborate distillation apparatus required. Dahlberg & Kosikowsky (704) were unable to establish a consistent relationship between flavour intensity of Cheddar cheese and pH, total volatile acidity or soluble protein content, though the latter was related to age. In Camembert cheese, on the other hand, flavour intensity was related to soluble protein and pH.

Methods for the fractionation of the nitrogenous compounds in cheese, for the study of protein breakdown, have been described and discussed in (61, 705, 706, 707). Sandelin (708) describes a method for following the decomposition of paracasein during cheese ripening which can be used to estimate the maturity of hard cheese.

Arbuckle (709) has used the mercurimetric chloride estimation with cheese. Schwarz & Fischer (710) have applied to cheese rapid methods originally evolved for the estimation of iron and copper in butter. A micro-method for zinc in cheese is described by Seuss (711). Effern (712) has estimated the zinc content of cheese by applying a dithizone extraction procedure to an acid solution of cheese ash. Values are given for the zinc content of the rind and interior of several varieties of cheese. Csiszár & Bakos (713) have suggested the minimum volume of ether extract which on evaporation yields visible crystals of benzoic acid as a rapid and approximate estimate of the benzoic acid content of processed cheese.

Olsson (714) has dealt with methods for determining moisture in whey products. Lawrence (715) has found a linear relationship between the total solids content and the specific gravity of rennet whey.

(*d*) *Dried milk, etc.*

Cone & Ashworth (716) have provided a method for determining the solubility of highly soluble milk powders which cannot be dealt with effectively by the method of the American Dry Milk Institute. By replacing the vigorous mechanical stirring of the American Dry Milk Institute method with a standardized and more gentle shaking procedure, a smaller proportion of the less soluble material is taken into solution and an increased sensitivity is obtained.

Chapman & McFarlane (717) have developed a method for the determination of the peroxide value of the fat in milk powders by treating an acetone solution of the fat with a reagent consisting of 0·1% of ferrous sulphate and 0·4% of ammonium thiocyanate in 96% acetone. The peroxide oxidizes its equivalent of ferrous iron and the red colour produced with the thiocyanate is measured. This technique was found to be more sensitive than the usual iodometric procedures. However, Lea (718) reports that this method is seriously influenced by the presence of atmospheric or dissolved oxygen. Loftus-Hills & Thiel (719), in a modification of the method, have eliminated this interference by replacing the 96% acetone with a mixture of benzene and methanol (70:30). Later, Loftus-Hills & Wilkinson (720) have shown that phosphatide extracted by the benzene-methanol solvent interferes with colour production when present in concentrations greater than 5 μg./10 ml. of extract. With phosphatide-rich powders, e.g. buttermilk powder, it is necessary to reduce the amount of powder to overcome this difficulty.

Two methods for the estimation of the brown colour in dried whey and skim milk have been described by Doob *et al.*(364). The first, applicable to whey only, involves the clarification of a suspension of the powder with alcoholic zinc chloride at 50° C. In the other, which may also be applied to dried skim milk, the powder is extracted at room temperature with a solution containing 300 g. of sodium chloride and 10 g. of trisodium phosphate per litre. The latter method extracts more colour from dried whey. Jenness, Coulter & Larson(721) have fractionated the fluorescing substances in dried milk by successive extraction of the powder with acetone-water (2:1), acetone-ether (1:4), and 10% potassium chloride solution. The fluorescing substances extractable by acetone-water and potassium chloride increased during storage, and the development of fluorescence was related to stale flavour development. Pyenson & Tracy(722) believe that, though chemical methods have been improved, organoleptic tests remain more reliable than peroxide or other chemical methods for determining the degree of deterioration in dried milk.

Methods for the estimation of reducing groups in processed milks and milk powders, based on the reduction of potassium ferricyanide(723) and of aneurin disulphide(724), have been evolved by Chapman & McFarlane and Harland & Ashworth. Both methods have been applied by the authors to estimations of the reducing groups of milks and milk powders subjected to various heat treatments.

Muers & Anderson(725) have described an ebullition and a more satisfactory density method for the estimation of air contained in particles of dried milk powder. Lea & Gane(726) have measured the density of skim milk and whole-milk powders, and have suggested the use of a density value of 1·33 to calculate the amount of enclosed air in a can of whole milk powder. A puncture unit connected to an evacuated burette, of use in the sampling and measurement of gases in food containers, has been described by Cartwright(727).

Extraction procedures for the estimation of fat in powdered foods have been described by Grossfeld & Schroeder(728) and Terrier(729). Schloemer & Catravas(730) have suggested that anhydrous, amorphous lactose may have a protective action in the extraction of fat from spray dried milk.

A method for the determination of ash in milk powder has been described by Schwarz & Hagemann(731). Its possible usefulness in the detection of added neutralizer has been examined by Schwarz & Mumm(732). On the basis of successful studies, the Tillmans-Bohrmann method for the determination of the alkalinity of ash has been adopted by the A.O.A.C. as a tentative method for the detection of added neutralizer(733). The method has proved fairly satisfactory,(734) but, as shown by Kunkel & Combs(735, 736), who applied this method to a large number of samples of dried skim milk and dried buttermilk, normal variation in ash alkalinity rules out the detection of small quantities of added neutralizer. Coulter & Kunkel(737) obtained a high correlation between the ash alkalinity of dried buttermilk and the electrical conductivity of the reconstituted product. Hillig's colorimetric method for the estimation of lactic acid [*J. Ass. off. agric. Chem., Wash.*, **20**, 130 (1937)] has given satisfactory results in a collaborative study of two samples of dried skim milk, one neutralized and one unneutralized(738). It is recommended that the method should be adopted tentatively.

Stamberg & Bolin(739) have described a rapid wet digestion method for the determination of calcium in biological materials. Figures obtained for dried skim milk were 5–6% higher than when dry ashing was employed to remove organic matter. Menefee(740) and Hetrick & Tracy(741) have determined copper in milk powder colorimetrically by the direct addition of citric acid, ammonia and carbonate to a solution of the ash in hydrochloric acid. Boulet & McFarlane(742) obtained a separation of copper from substances likely to interfere in its estimation by refluxing milk powder with potassium thiocyanate in 95% acetone. This necessitates an alcohol extraction of carotenoids after colour development. Morrison & Paige(743) have modified the method of Bendix & Grabenstetter [*Industr. Engng Chem.* (Anal. ed.), **15**, 649 (1943)] for the estimation of copper in dried milk. Wet digestion and ashing techniques have been given by Thompson(744) and Pyenson & Tracy(745) respectively, for the separation of iron from milk powders previous to its colorimetric estimation.

Using the toluene distillation method, Choi, O'Malley & Fairbanks(746) found dehydration of

α-lactose to be a reaction of the first order and the rate to vary inversely with the size of the particles, factors of importance in moisture determinations in dried products.

Suitable modifications of the Babcock method for the estimation of fat in concentrated milk, chocolate milk and ice cream (747), concentrated milk (748), and sweetened condensed and evaporated milk (749) have been described. A modified Gerber method, sufficiently accurate for the estimation of the fat content of sweetened condensed milk for rapid control purposes, has been provided by Stanworth (750). Details of the standardization of milk sweetened before condensing are also given. Steam and vacuum distillation have been compared by Miller, Zimmerman & Oberg (751) for the separation of volatile fatty acids from evaporated milk.

Ice-cream literature has been adequately covered in a series of reviews published by Leighton (752).

National Institute for Research in Dairying
University of Reading

R. Aschaffenburg
J. A. F. Rook

REFERENCES

(1) Rowland & Scott Blair (1942). *J. Dairy Res.* **13**, 93.
(2) Houdinière (1944). *Lait*, **24**, 108, 226, 313; (1945). **25**, 27, 97.
(3) Mukherjee, Swaminathan & Viswanath (1944). *Indian J. vet. Sci.* **14**, 232.
(4) Riddet, Campbell, McDowall & Cox (1941–42). *N.Z. J. Sci. Tech.* **23**, A, 80.
(5) Rowland (1944). *J. Dairy Res.* **13**, 261.
(6) Rowland (1946). *Dairy Industr.* **11**, 656.
(7) Davel (1947). *Fmg S. Afr.* **22**, 242.
(8) Bakalor (1947). *Fmg S. Afr.* **22**, 807.
(9) Rensburg, van (1946). *Fmg S. Afr.* **21**, 217.
(10) Purchase & Reverberi (1946). *E. Afr. agric. J.* **12**, 50.
(11) Herman & Peterson (1942). *Rep. Mo. agric. Exp. Sta.* 1938–9, (*Bull.* no. 444), p. 35.
(12) Amaral Rogick (1943). *Bol. Industr. Anim.* **6**, (3), 59; (1946), **8**, (1/2), 102 and (3), 84.
(13) Mork (1942). *Meld. Norg. LandbrHogsk. MeieriAvd.* no. 21.
(14) Kästli (1945). *Schweiz. Milchztg*, **71**, 291, 295, 299, 303.
(15) Kästli & Binz (1946). *Schweiz. Milchztg*, **72**, (96).
(16) Mosimann (1944). *Mitt. Lebensm. Hyg., Bern*, **35**, 14.
(17) Mosimann (1944). *Schweiz. Milchztg*, **70**, 311.
(18) Kästli (1940). *Mitt. Lebensm. Hyg., Bern*, **31**, 46.
(19) Thomé (1944). *Medd. Statens Mejeriförsök.* no. 14.
(20) McClymont & Paxton (1947). *Agric. Gaz. N.S.W.* **58**, 551.
(21) Leroy & Bonnet (1947). *Ann. agron., Paris*, **17**, 454.
(22) Filipovič (1942). *Milchw. Forsch.* **21**, 179.
(23) Filipovič (1942). *Milchw. Forsch.* **21**, 206.
(24) Mikič (1942). *Milchw. Forsch.* **21**, 190.
(25) Davis & Harland (1946). *J. Dairy Sci.* **29**, 839.
(26) Miller & Wise (1944). *J. Dairy Sci.* **27**, 275, 281, 287, 293.
(27) Heinemann (1947). *J. Dairy Sci.* **30**, 757.
(28) Yska (1947). *Off. Org. algem. ned. Zuivelb.* **39**, (13), 103.
(29) Koestler, Kägi, Lehmann & Wegmüller (1944). *Annu. agric. Suisse*, **45**, 107.
(30) Rees (1947). *Aust. J. Dairy Tech.* **2**, 3.
(31) Overman (1945). *J. Dairy Sci.* **28**, 305.
(32) Moore & Morrow (1941/42). *Rep. N.H. agric. Exp. Sta.* (*Bull.* no. 345), p. 19.
(33) Bonnier, Hansson & Jarl (1946). *Acta Agric. suec.* **2**, 159.
(34) Neseni & Körprich (1947). *Milchwissenschaft*, **2**, 389, 405.
(35) Noll & Supplee (1941). *J. Dairy Sci.* **24**, 993.
(36) Ghosh & Datta Roy (1941). *Indian med. Gaz.* **76**, 279.
(37) Anantakrishnan, Dastur & Kothavalla (1944). *Indian J. vet. Sci.* **13**, 297.
(38) Holmes, Kuzmeski, Lindquist & Rodman (1946). *Amer. J. Dis. Child.* **71**, 647.

(39) Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (1947). *Brit. J. Nutrit.* **1**, 64.
(40) Holmes, Spelman, Smith & Kuzmeski (1947). *J. Dairy Sci.* **30**, 385.
(41) Adriani, Tamsma, Vogel & Groot (1945). *Onderzoek naar de factoren die de samenstelling van het melkvet beïnvloeden.* The Hague: Vakgroep Boterindustrie.
(42) Sandelin (1939). *Meijerit. Aikakausk.* **1**, 111.
(43) Thiel (1943). *J. Coun. sci. industr. Res. Aust.* **16**, 139.
(44) Sörensen (1946). *Aarsberetn. St. Smørbedømm.* 1945, p. 33.
(45) Storgårds (1945). *Suom. Kem.* **18A**, (1/2), 26.
(46) Caserio (1940). *Ann. Igiene*, **50**, 453.
(47) Angelescu (1941/42). *Bul. Chim. pur. apl.*, Ser. 2, **3**, Sect. A, 68.
(48) Taha El-Katib (1946). *Nature, Lond.*, **158**, 747.
(49) Schwarz & Mumm (1942). *Molkereiztg, Hildesh.*, **56**, 401.
(50) Koestler (1944). *Schweiz. Milchztg*, **70**, 395.
(51) Zollikofer (1942). *Schweiz. Milchztg*, **68**, 58.
(52) Csiszár, Bakos & Tomka (1942). *Milchw. Forsch.* **21**, 236.
(53) Sproule (1943). *Canad. Dairy Ice Cr. J.* **22**, (2), 27.
(54) Irvine, Bryant, Sproule, Jackson, Crook & Johnstone (1945). *Sci. Agric.* **25**, 817.
(55) Irvine, Bryant, Sproule, Jackson, Crook & Johnstone (1945). *Sci. Agric.* **25**, 833.
(56) Irvine, Bryant, Sproule, Jackson, Crook & Johnstone (1945). *Sci. Agric.* **25**, 845.
(57) Garrett (1943). *J. Dairy Sci.* **26**, 305.
(58) Zahrndt, Lane & Hammer (1944). *Res. Bull. Ia agric. Exp. Sta.* no. 325, p. 144.
(59) Schulz (1946). *Milchwissenschaft*, **1**, 7.
(60) Hoecker & Hammer (1943). *J. Dairy Sci.* **26**, 772.
(61) Sirks (1943). *Versl. Ver. Proefzuiv. Hoorn*, 1942, p. 99.
(62) Ashworth & Van Orden (1943). *J. Milk Tech.* **6**, 272.
(63) Heinemann (1944). *J. Dairy Sci.* **27**, 773.
(64) Gould & Skiver (1945). *Quart. Bull. Mich. agric. Exp. Sta.* **27**, 360.
(65) Acharya & Devadatta (1941). *J. Univ. Bombay*, **9**, part 5, 1.
(66) Anantakrishnan & Kothavalla (1946). *Indian J. vet. Sci.* **16**, 56.
(67) Anantakrishnan, Bhale & Paul (1946). *Biochem. J.* **40**, 292.
(68) Baldwin & Longenecker (1944). *J. biol. Chem.* **155**, 407.
(69) Hilditch & Jasperson (1945). *J. Soc. chem. Ind., Lond.*, **64**, 109.
(70) Brouwer & Jonker-Scheffener (1946). *Rec. Trav. chim. Pays-Bas*, **65**, 408.
(71) Geyer, Nath, Barki, Elvehjem & Hart (1947). *J. biol. Chem.* **169**, 227.
(72) Mulder (1945). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 51 (2) C, 39.
(73) Jack & Henderson (1945). *J. Dairy Sci.* **28**, 65.
(74) Jack, Henderson & Hinshaw (1946). *J. biol. Chem.* **162**, 119.
(75) Gould (1947). *J. Dairy Sci.* **30**, 901.
(76) Hilditch & Jasperson (1943). *Biochem. J.* **37**, 238.
(77) Sjollema (1943). *Onderzoekingen over de oorzaken van afwijkende stevigheid van boter bij weidegang.* Rotterdam: Cent. Bur. Ned. Landb. Com.
(78) Winther (1944). *Beretn. Forsøgs-lab., Kbh.*, no. 208.
(79) *Rep. Coun. sci. industr. Res. Aust.* 1940–1, p. 74.
(80) Zollikofer (1941). *Schweiz. Milchztg*, **67**, (21), 95; (22), 97.
(81) Schloemer (1940). *Z. Untersuch. Lebensmitt.* **79**, 561.
(82) Rauch & Schloemer (1940). *Z. Untersuch. Lebensmitt.* **80**, 243.
(83) Van der Burg, Brouwer & Koppejan (1944). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 50 (1) G, 1.
(84) Fitelson (1943). *J. Ass. off. agric. Chem., Wash.*, **26**, 506.
(85) Hoecker & Hammer (1942). *J. Dairy Sci.* **25**, 175.
(86) Hedrick & Hammer (1942). *Res. Bull. Ia agric. Exp. Sta.* no. 301, p. 828.
(87) Hedrick (1942). *Iowa St. Coll. J. Sci.* **17**, 77.
(88) Hilditch & Meara (1944). *Biochem. J.* **38**, 29.
(89) Baldwin & Longenecker (1944). *J. biol. Chem.* **154**, 255.
(90) Brown & Orians (1946). *Arch. Biochem.* **9**, 201.

(91) Hilditch & Meara (1944). *Biochem. J.* **38**, 437.
(92) Anantakrishnan, Bhale, Paul & Rangaswamy (1946). *J. biol. Chem.* **166**, 31.
(93) Achaya & Banerjee (1945). *Indian J. vet. Sci.* **15**, 271.
(94) Achaya & Banerjee (1946). *Biochem. J.* **40**, 664.
(95) Achaya & Banerjee (1946). *Curr. Sci.* **15**, 23.
(96) Hilditch & Jasperson (1944). *Biochem. J.* **38**, 443.
(97) de la Mare & Shorland (1944). *Nature, Lond.*, **153**, 380.
(98) Zeisset & Grossfeld (1942). *Z. Untersuch. Lebensmitt.* **83**, 385.
(99) Chibnall (1942). *Proc. roy. Soc.* B, **131**, (863), 136.
(100) Chibnall (1946). *J. int. Soc. Leath. Chem.* **30**, 1.
(101) Chibnall, Rees & Williams (1943). *Biochem. J.* **37**, 354.
(102) Bolling & Block (1943). *Arch. Biochem.* **2**, 93.
(103) Chibnall, Rees & Williams (1943). *Biochem. J.* **37**, 372.
(104) Tristram (1946). *Biochem. J.* **40**, 72.
(105) Rees (1946). *Biochem. J.* **40**, 632.
(106) Schweigert (1947). *J. Nutrit.* **33**, 553.
(107) Bergdoll & Doty (1946). *Industr. Engng Chem.* (Anal. ed.), **18**, 600.
(108) Gordon, Semmett, Cable & Doherty (1947). *Fed. Proc.* **6**, 255.
(109) Nicolet, Shinn & Saidel (1942). *Abstr. Pap. Amer. chem. Soc.* p. 22B.
(110) Goodwin & Morton (1946). *Biochem. J.* **40**, 628.
(111) Dunn & Rockland (1946). *Arch. Biochem.* **11**, 89.
(112) Williamson (1944). *J. biol. Chem.* **156**, 47.
(113) Kugenev (1946). *Dokl. mosk. sel.-khoz. Akad. Timiryazeva*, no. III, p. 149.
(114) Anantakrishnan & Lahiry (1942). *Indian J. med. Res.* **30**, 433.
(115) Waisman & Petazzo (1945). *Rev. Asoc. argent. Dietol.* **3**, (11), 166.
(116) Karabinos & Dittmer (1943). *Proc. Soc. exp. Biol., N.Y.*, **53**, 111.
(117) Willstaedt, Borggard & Lieck (1946). *Z. Vitaminforsch.* **18**, 25.
(118) Cohen (1943). *Arch. Biochem.* **2**, 345.
(119) Frost & Heinsen (1945). *J. biol. Chem.* **161**, 517.
(120) Schein & Berg (1946). *Arch. Biochem.* **11**, 209.
(121) Feigenbaum (1945). *Exp. med. Surg.* **3**, (1), 24.
(122) Hoover & Kokes (1947). *J. biol. Chem.* **167**, 199.
(123) Winnick (1944). *J. biol. Chem.* **152**, 465.
(124) Cheesman & Ehrensvärd (1945). *Ark. Kemi Min. Geol.*, **19**A, (20), 1; **21**B, (1) 1.
(125) Maass (1947). *Milchwissenschaft*, **2**, 286.
(126) Haugaard & Roberts (1942). *J. Amer. chem. Soc.* **64**, 2664.
(127) Cole & Tarassuk (1946). *J. Dairy Sci.* **29**, 421.
(128) Sandelin (1945). *Meijerit. Aikakausk.* **7**, 11.
(129) Sørensen & Sørensen (1939). *C.R. Lab. Carlsberg*, **23**, (7), 55.
(130) Harland & Ashworth (1945). *J. Dairy Sci.* **28**, 87.
(131) Dills & Nelson (1942). *J. Amer. chem. Soc.* **64**, 1616.
(132) Pino (1947). *Lait*, **27**, 113.
(133) Decker & Reid (1943). *Res. Bull. Mo. agric. Exp. Sta.* no. 373.
(134) Aas (1941). *Meieriposten*, **30**, 763.
(135) Leviton (1943). *Industr. Engng Chem.* (Industr. ed.), **35**, 589.
(136) Leviton (1944). *Industr. Engng Chem.* (Industr. ed.), **36**, 744.
(137) Semmons & McHenry (1946). *Canad. J. publ. Hlth*, **37**, 22.
(138) Robinson & Hlynka (1947). *Canad. J. publ. Hlth*, **38**, 95.
(139) Seekles & Smeets (1947). *Neth. Milk Dairy. J.* **1**, 7.
(140) Sandelin (1941). *Meijerit. Aikakausk.* **3**, (1), 38.
(141) Basu & Mukherjee (1943). *Indian J. vet. Sci.* **13**, 231.
(142) Sandelin (1945). *Meijerit. Aikakausk.* **7**, (1/2), 1.
(143) White, Clifford & Calvery (1943). *J. Amer. vet. med. Ass.* **102**, 292.
(144) Stas (1941). *Pharm. Weekbl.* **78**, 25.
(145) Luecke, Duncan & Ely (1947). *Arch. Biochem.* **13**, 277.

(146) Holland & Ritchie (1941). *Bull. Mass. agric. Exp. Sta.* no. 379.
(147) Plumier (1946). *C.R. Soc. Biol., Paris*, **140**, 680, 682.
(148) Pohle, Cook & Mehlenbacher (1947). *Food Res.* **12**, 229.
(149) Itzerott (1942). *J. Aust. Inst. agric. Sci.* **8**, 119.
(150) Schwaibold & Lesmüller (1941). *Vorratspfl. u Lebensmittelforsch.* **4**, 100.
(151) Archibald (1947). *J. Dairy Sci.* **30**, 293.
(152) Venn, McCance & Widdowson (1947). *J. comp. Path.* **57**, 314.
(153) Sjöström (1940). *Svensk Kem. Tidskr.* **52**, 310.
(154) Crane & Horrall (1943). *J. Dairy Sci.* **26**, 935.
(155) Kahane & Lévy (1945). *Lait*, **25**, 193.
(156) Townley & Gould (1943). *J. Dairy Sci.* **26**, 843, 853.
(157) Townley & Gould (1943). *Quart. Bull. Mich. agric. Exp. Sta.* **26**, 52.
(158) Gould & Frantz (1946). *J. Dairy Sci.* **29**, 27.
(159) Howell, Cave, Heller & Gross (1947). *J. Dairy Sci.* **30**, 717.
(160) Caputto & Marsal (1941). *Rev. Soc. argent. Biol.* **17**, 139.
(161) Caputto & Marsal (1944). *Rev. Fac. Cienc. med. Córdoba*, **2**, (2), 3; (12), 15.
(162) Kelly (1941). *Rep. Bur. Dairy Ind., U.S. Dep. Agric.* 1940, p. 48.
(163) Massart & Vandendriessche (1945). *Enzymologia*, **11**, 261.
(164) Sizer (1940). *Anat. Rec.* **81**, (4), Suppl. p. 120.
(165) Bever, van (1943). *Enzymologia*, **11**, 7.
(166) Bever, van & Straub (1941). *Chem. Weekbl.* **38**, 210.
(167) Stijgeren, van (1943). *Hand. Genoot. Melkkunde*, **2**.
(168) Hartmann & Andersen (1943). *Aarsberetn. Forsøgsm., Kbh.*, 1942–3, Beretn. no. 38, p. 15.
(169) Sanders & Sager (1947). *J. Dairy Sci.* **30**, 518.
(170) Sjöström (1942). *Svenska Mejeritidn.* **34**, 257.
(171) Guittonneau, Chevalier & Jarrousse (1944). *C.R. Acad. Sci., Paris*, **218**, 1006.
(172) Ferraz Lopes & Soares Filho (1944). *Bol. Industr. anim.*, **7**, 47.
(173) Lythgoe (1942). *J. Dairy Sci.* **25**, 961.
(174) Hershey, Waldrip & Willett (1943). *Amer. J. publ. Hlth*, **33**, 339.
(175) Fischer (1942). *Milchw. Forsch.* **21**, 242.
(176) Kay (1943). *Dairy Industr.* **8**, 688.
(177) Burgwald (1942). *J. Dairy Sci.* **25**, 285.
(178) Stiven (1947). *J. Dairy Res.* **15**, 57.
(179) Scharer (1943). *Amer. J. publ. Hlth*, **33**, 396.
(180) Andersen & Hartmann (1942). *Aarsberetn. Forsøgsm., Kbh.*, 1941–2, Beretn. no. 34, p. 20.
(181) Hartmann (1942). *Aarsberetn. Forsøgsm., Kbh.*, 1941–2, Beretn. no. 34, p. 26.
(182) Andersen & Vestesen (1944). *Beretn. Forsøgslab., Kbh.*, no. 210.
(183) Horwitz (1946). *J. Ass. off. agric. Chem., Wash.*, **29**, 129.
(184) Sanders & Sager (1946). *J. Dairy Sci.* **29**, 737.
(185) Aschaffenburg (1947). *Mon. Bull. Minist. Hlth Lab. Serv., Lond.*, **6**, 159.
(186) Andersen (1944). *Oversigt Forsøgslab. Arbejder, Kbh.*, 1943–4, no. 68.
(187) Thomé (1941). *Medd. Statens Mejeriförsök.* no. 6.
(188) Brown & Elliker (1942). *J. Bact.* **43**, 118.
(189) Barber & Frazier (1943). *J. Dairy Sci.* **26**, 343.
(190) Scharer (1945). *Amer. J. publ. Hlth*, **35**, 358.
(191) Caulfield & Martin (1945). *J. Dairy Sci.* **28**, 155.
(192) Gilcreas (1947). *J. Ass. off. agric. Chem., Wash.*, **30**, 422.
(193) Horwitz (1947). *J. Ass. off. agric. Chem., Wash.*, **30**, 430.
(194) Sanders & Sager (1947). *J. Dairy Sci.* **30**, 909.
(195) Hankinson (1942). *J. Dairy Sci.* **25**, 727.
(196) Hankinson (1943). *J. Dairy Sci.* **26**, 53.
(197) Berridge (1943). *Nature, Lond.*, **151**, 473.
(198) Berridge (1945). *Biochem. J.* **39**, 179.
(199) Hankinson & Palmer (1943). *J. Dairy Sci.* **26**, 1043.
(200) Pyne (1945). *Biochem. J.* **39**, 385.

(201) Moriyama (1940). *Arch. ges. Virusforsch.* **1**, 510.
(202) Moriyama (1941). *Arch. ges. Virusforsch.* **2**, 71.
(203) Moriyama & Ohashi (1941). *Naturwissenschaften*, **29**, 334.
(204) Mazé (1940). *C.R. Soc. Biol., Paris*, **134**, 425.
(205) Mazé & Mazé, Jr. (1941). *C.R. Soc. Biol., Paris*, **135**, 808.
(206) Vilegzhanin (1942). *Proc. Vologda agric. Inst.* 1942, v, 275.
(207) Tapernoux (1942). *C.R. Soc. Biol., Paris*, **136**, 816.
(208) Peterson, Johnson & Price (1943). *J. Dairy Sci.* **26**, 233.
(209) Krukovsky & Herrington (1942). *J. Dairy Sci.* **25**, 231.
(210) Krukovsky & Herrington (1942). *J. Dairy Sci.* **25**, 237.
(211) Herrington & Krukovsky (1942). *J. Dairy Sci.* **25**, 241.
(212) Kay (1946). *Nature, Lond.*, **157**, 511.
(213) Tarassuk & Henderson (1942). *J. Dairy Sci.* **25**, 801.
(214) Hlynka & Hood (1942). *J. Dairy Sci.* **25**, 389.
(215) Kelly (1945). *J. Dairy Sci.* **28**, 803.
(216) Kelly (1944). *J. Dairy Sci.* **27**, 644.
(217) Gould (1941). *J. Dairy Sci.* **24**, 779.
(218) Gould (1942). *J. Dairy Sci.* **25**, 869.
(219) Jack, Tarassuk & Scaramella (1942). *Nat. Butt. Cheese J.* **33**, (12), 16.
(220) Hlynka, Hood & Gibson (1942). *J. Dairy Sci.* **25**, 923.
(221) Babel & Hammer (1943). *J. Dairy Sci.* **26**, 773.
(222) Lythgoe & Rosenthal (1942). *Abstr. Pap. Amer. chem. Soc.* **104**, 16A.
(223) Theorell & Åkeson (1943). *Ark. Kemi Min. Geol.* **17**B, (7), 1.
(224) Theorell & Paul (1945). *Ark. Kemi Min. Geol.* **18**A, (12), 1.
(225) Theorell & Pedersen (1944). *The Svedberg*, 1884–1944, p. 523, Uppsala.
(226) Fischer (1943). *Milchw. Forsch.* **21**, 285.
(227) Schwarz & Fischer (1942). *Milchw. Forsch.* **21**, 161.
(228) Kreker (1939). *Schr. Reichskurat. Tech. Landw.* no. 92.
(229) Diemair & Hausser (1941). *Z. anal. Chem.* **122**, 12, 173.
(230) Pien (1945). *Lait*, **25**, 311.
(231) Schwarz & Sydow (1947). *Milchwissenschaft*, **2**, 424.
(232) Schönberg & Milbradt (1942). *Dtsch. Tierärztl. Wschr.* **50**, (5/6), 69.
(233) Schellhase (1944). *Berl. Münch. tierärztl. Wschr.* 1944 (9–10), p. 75.
(234) Schloemer & Bleyer (1940). *Z. Untersuch. Lebensmitt.* **80**, 425.
(235) Worden (1943). *Nature, Lond.*, **152**, 505.
(236) Polonovski, Neuzil, Baudu & Polonovski (1947). *C.R. Soc. Biol., Paris*, **141**, 460.
(237) Araujo (1942). *Rev. Brasil. Biol.* **2**, 53.
(238) Keston (1944). *J. biol. Chem.* **153**, 335.
(239) Warner & Polis (1945). *J. Amer. chem. Soc.* **67**, 529.
(240) Vilstrup & Bielefeldt (1940). *Bull. Off. int. Choc. Cacao, Brux.*, **10**, (1), 3.
(241) Warner (1944). *J. Amer. chem. Soc.* **66**, 1725.
(242) Krejci, Jennings & Smith (1941). *J. Franklin Inst.* **232**, 592.
(243) Nitschmann & Lehmann (1947). *Experientia*, **3**, (4).
(244) Mellander (1945). *Nature, Lond.*, **155**, 604.
(245) Chanutin, Ludewig & Masket (1942). *J. biol. Chem.* **143**, 737.
(246) Klotz (1946). *Arch. Biochem.* **9**. 109.
(247) Eilers (1945). *Versl. landb. Onderzoek. Minist. Landb., 's Grav.*, no. 50 (15) G, 1009; English version in: *Monographs on the Progress of Research in Holland during the War*, no. 12. New York and Amsterdam: Elsevier Publ. Co. Inc. (1947).
(248) de Kadt & Minnen (1943). *Rec. Trav. chim. Pays-Bas*, **62**, 257.
(249) Burg, van der (1947). *Neth. Milk Dairy J.* **1**, 69.
(250) Ramsdell & Whittier (1944). *J. biol. Chem.* **154**, 413.
(251) Sandelin (1943). *Meijerit. Aikakausk.* **5**, 1.
(252) Sandelin (1943). *Svenska Mejeritidn.* **35**, (18), (19), (20).
(253) Küntzel & Doehner (1940). *Kolloidbeihefte*, **51**, 277.

(254) Lotmar & Nitschmann (1941). *Helv. chim. Acta*, **24**, 242.
(255) Jirgensons (1942). *J. prakt. Chem.* **160**, 120.
(256) Jirgensons (1943). *J. prakt. Chem.* **162**, 224.
(257) Rao, Doss & Rao (1946). *Proc. Ind. Acad. Sci.* **23**, A, 8.
(258) Rao, Doss & Rao (1946). *Proc. Ind. Acad. Sci.* **24**, A, 277.
(259) Erdös (1942). *Stud. Inst. med. Chem. Univ. Szeged*, **2**, 37.
(260) Laki (1942). *Stud. Inst. med. Chem. Univ. Szeged*, **2**, 43.
(261) Jirgensons (1942). *Kolloidzschr.* **99**, 314.
(262) Mecham & Olcott (1947). *Industr. Engng Chem.* (Industr. ed.), **39**, 1023.
(263) Nitschmann & Lehmann (1947). *Helv. chim. Acta*, **30**, 804.
(264) Mellander (1947). *Uppsala LäkFören. Förh.* **52**, 108.
(265) Olcott & Fraenkel-Conrat (1946). *Industr. Engng Chem.* (Industr. ed.), **38**, 104.
(266) Mellon, Korn & Hoover (1947). *J. Amer. chem. Soc.* **69**, 827.
(267) French & Edsall (1945). *Advances in Protein Chemistry*, **2**, 1277.
(268) Nitschmann & Hadorn (1941). *Helv. chim. Acta*, **24**, 237.
(269) Nitschmann, Hadorn & Lauener (1943). *Helv. chim. Acta*, **26**, 1069.
(270) Nitschmann & Lauener (1946). *Helv. chim. Acta*, **29**, 174.
(271) Nitschmann & Lauener (1946). *Helv. chim. Acta*, **29**, 180.
(272) Nitschmann & Hadorn (1943). *Helv. chim. Acta*, **26**, 1075.
(273) Nitschmann & Hadorn (1943). *Helv. chim. Acta*, **26**, 1084.
(274) Nitschmann & Hadorn (1944). *Helv. chim. Acta*, **27**, 299.
(275) Nitschmann & Lauener (1946). *Helv. chim. Acta*, **29**, 184.
(276) Carpenter & Lovelace (1942). *Industr. Engng Chem.* (Industr. ed.), **34**, 759.
(277) Carpenter & Lovelace (1944). *Industr. Engng Chem.* (Industr. ed.), **36**, 680.
(278) Wormell & Kaye (1944). *Nature, Lond.*, **153**, 525.
(279) Wormell & Kaye (1945). *J. Soc. chem. Ind., Lond.*, **64**, 75.
(280) Fraenkel-Conrat, Cooper & Olcott (1945). *J. Amer. chem. Soc.* **67**, 950.
(281) Brand & Kassell (1942). *Fed. Proc.* **1**, (1), part II, 103.
(282) Brand & Kassell (1942). *J. biol. Chem.* **145**, 365.
(283) Gutfreund (1945). *Nature, Lond.*, **155**, 237.
(284) Bull & Currie (1946). *J. Amer. chem. Soc.* **68**, 742.
(285) Bull (1945). *J. Amer. chem. Soc.* **67**, 8.
(286) Bull (1946). *J. Amer. chem. Soc.* **68**, 745.
(287) Fankuchen (1942). *J. Amer. chem. Soc.* **64**, 2504.
(288) McMeekin & Warner (1942). *J. Amer. chem. Soc.* **64**, 2393.
(289) Gorin & Moyer (1942). *J. gen. Physiol.* **25**, 785.
(290) Briggs & Hanig (1944). *J. phys. chem.* **48**, 1.
(291) Li (1946). *J. Amer. chem. Soc.* **68**, 2746.
(292) Cannan, Palmer & Kibrick (1942). *J. biol. Chem.* **142**, 803.
(293) Grönwall (1942). *C.R. Lab. Carlsberg* (Sér. chim.), **24**, 185.
(294) Grönwall (1942). *C.R. Lab. Carlsberg* (Sér. chim.), **24**, 201.
(295) Grönwall (1942). *C.R. Lab. Carlsberg* (Sér. chim.) **24**, 217.
(296) Briggs & Hull (1945). *J. Amer. chem. Soc.* **67**, 2007.
(297) Spremulli (1942). *Univ. Microfilms Publ.* no. 510. *Abstr.Doc. Dissert., Pa St. Coll.* **5**, 20.
(298) Miller (1939). *C.R. Lab. Carlsberg* (Sér. chim.), **23**, (6), 45.
(299) Bull (1944). *J. Amer. chem. Soc.* **66**, 1499.
(300) Pauling (1945). *J. Amer. chem. Soc.* **67**, 555.
(301) Smith (1946). *J. biol. Chem.* **164**, 345.
(302) Smith & Coy (1946). *J. biol. Chem.* **164**, 367.
(303) Smith (1946). *J. biol. Chem.* **165**, 66.
(304) Deutsch (1947). *J. biol. Chem.* **169**, 437.
(305) Grönwall (1947). *Nature, Lond.*, **159**, 376.
(306) Hansen, Phillips, Williams & Smith (1946). *J. Dairy Sci.* **29**, 521.
(307) Aschaffenburg (1946). *J. Dairy Res.* **14**, 316.
(308) El-Rafey & Richardson (1944). *J. Dairy Sci.* **27**, 1.

(309) Sommer (1943). *J. Dairy Sci.* **26**, 759.
(310) Palmer (1944). *J. Dairy Sci.* **27**, 47.
(311) Jenness & Palmer (1945). *J. Dairy Sci.* **28**, 611.
(312) Sandelin (1941). *Meijerit. Aikakausk.* **3**, 1.
(313) Sandelin (1945). *Meijerit. Aikakausk.* **7**, 17.
(314) Skelton (1943). *Rep. S. Dak. agric. Exp. Sta.* 1942–3.
(315) Dunkley & Sommer (1944). *Res. Bull. Wis. agric. Exp. Sta.* no. 151.
(316) Skelton & Sommer (1944). *J. Dairy Sci.* **27**, 321.
(317) Peters & Trout (1945). *J. Dairy Sci.* **28**, 277.
(318) Peters & Trout (1945). *J. Dairy Sci.* **28**, 283.
(319) Straub & Bever, van (1941). *Handb. Genoot. Mekkunde*, 1941, I.
(320) Leviton & Haller (1947). *J. phys. Colloid Chem.* **51**, 460.
(321) Mocquot (1945). *C.R. Acad. Sci., Paris*, **221**, 121.
(322) Occhialini (1942). *Ann. Chim. appl., Roma*, **32**, 110.
(323) Lyons (1946). *Econ. Proc. Roy. Dublin Soc.* **3**, 249.
(324) Mulder (1947). *Neth. Milk Dairy J.* **1**, 57.
(325) Mohr & Hennings (1947). *Milchwissenschaft*, **2**, 173.
(326) Trout & Scheid (1941). *Quart. Bull. Mich. agric. Exp. Sta.* **24**, 122.
(327) Babcock (1942). *Tech. Bull. U.S. Dep. Agric.* no. 832.
(328) Peters & Trout (1945). *Canad. Dairy Ice Cr. J.* **24**, (3), 25.
(329) Peters & Trout (1945). *J. Dairy Sci.* **28**, 251.
(330) Peters & Trout (1945). *J. Milk Tech.* **8**, 13.
(331) Steel (1940). *Proc. W. Div. Amer. Dairy Sci. Ass.* 1940, p. 12.
(332) Doan, Josephson & Adams (1941). *Milk Dlr*, **31**, (2), 35.
(333) Trout & Scheid (1942). *Quart. Bull. Mich. agric. Exp. Sta.* **25**, 22.
(334) Doan & Mykleby (1943). *J. Dairy Sci.* **26**, 893.
(335) Judkins (1943). *J. Dairy Sci.* **26**, 997.
(336) Whitaker (1944). *Ass. Bull. (Int. Ass. Milk Dlrs)*, **36**, 67.
(337) Farrall, Walts & Hanson (1941). *J. Dairy Sci.* **24**, 539.
(338) Snyder & Sommer (1943). *Milk Dlr*, **32**, (5), 36.
(339) Brasch & Huber (1947). *Science*, **105**, 112.
(340) Gould (1946). *Abstr. Pap. Amer. chem. Soc.*, **109**, 12A.
(341) Harland, Jenness & Coulter (1947). *J. Dairy Sci.* **30**, 526.
(342) Kass & Palmer (1942). *Abstr. Pap. Amer. chem. Soc.* **104**, 13A.
(343) Guittonneau, Bejambes, Veisseyre & de Lamothe (1946). *C.R. Acad. Sci., Paris*, **222**, 458.
(344) Josephson (1943). *Abstr. Doc. Dissert., Pa St. Coll.* **6**, 90.
(345) Josephson & Dahle (1945). *Food Industr.* **17**, 630.
(346) Ewbank & Gould (1943). *J. Dairy Sci.* **26**, 409.
(347) Lea (1946). *Analyst*, **71**, 227.
(348) Findlay, Higginbottom, Smith & Lea (1946). *J. Dairy Res.* **14**, 378.
(349) White, Smith & Lea (1947). *J. Dairy Res.* **15**, 127.
(350) Webb, Bell, Deysher & Holm (1943). *J. Dairy Sci.* **26**, 571.
(351) Bell & Webb (1943). *J. Dairy Sci.* **26**, 579.
(352) Webb & Bell (1943). *J. Dairy Sci.* **26**, 1071.
(353) Babcock, Roerig, Stabile, Dunlap & Randall (1946). *J. Dairy Sci.* **29**, 699.
(354) Babcock, Roerig, Stabile, Dunlap & Randall (1947). *J. Dairy Sci.* **30**, 49.
(355) Doan & Warren (1947). *J. Dairy Sci.* **30**, 837.
(356) Freeman, Mull & Fouts (1943). *Bull. Fla agric. Exp. Sta.* no. 383.
(357) Mohr & Eysank (1940). *Forschungsdienst*, **10**, 579.
(358) Greenbank, Wright & Deysher (1944). *J. Dairy Sci.* **27**, 686.
(359) Lea, Moran & Smith (1943). *J. Dairy Res.* **13**, 162.
(360) Coulter & Jenness (1945). *Tech. Bull. Minn. agric. Exp. Sta.* no. 167.
(361) Hetrick & Tracy (1944). *J. Dairy Sci.* **27**, 685.
(362) Greenbank, Wright, Deysher & Holm (1946). *J. Dairy Sci.* **29**, 55.
(363) Thiel (1945). *J. Coun. sci. industr. Res. Aust.* **18**, 391.
(364) Doob, Willmann & Sharp (1942). *Industr. Engng Chem.* (Industr. ed.), **34**, 1460.

(365) Webb, Deysher & Hufnagel (1946). *J. Dairy Sci.* **29**, 499.
(366) Gould & Leininger (1947). *Quart. Bull. Mich. agric. Exp. Sta.* **30**, 54.
(367) Romani (1944). *Chim. Industr., Milano*, **26**, 134.
(368) Gould, Weaver & Frantz (1946). *J. Dairy Sci.* **29**, 33.
(369) Loftus-Hills (1947). *J. Dairy Res.* **15**, 62.
(370) White (1947). *Canad. Dairy Ice Cr. J.* **26**, (6), 21.
(371) McDowall (1946). *N.Z. J. Sci. Tech.* **28**, A, 132.
(372) Bird (1941–42). *Rep. Ia agric. Exp. Sta.* 1940–1, I, 143; 1941–2, I, 163.
(373) Adriani & Tamsma (1941). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 47 (12) G, 941.
(374) Sandelin (1943). *Meijerit. Aikakausk.* **5**, 95.
(375) Mohr & Eysank (1944). *Fette u. Seifen*, **51**, 267.
(376) Fritz (1946). *Milchwissenschaft*, **1**, 2, 74.
(377) Imperial Dairy Research Institute, Bangalore (1945). *Indian Fmg*, **6**, 580.
(378) Linneboe (1943). *Sci. Agric.* **24**, 64.
(379) Richardson & Long (1947). *J. Dairy Sci.* **30**, 533.
(380) Spaght (1945). *Rep. Comb. Intell. Object. Sub-Comm.* file no. XXXI–79, item no. 22. London: H.M. Stationery Office.
(381) Hostettler (1942). *Schweiz. landw. Mh.* **20**, (8), 1.
(382) Scotland, Department of Health, Scientific Advisory Committee (1945). *The Freezing Point (Hortvet) Test of Milk.* Report of a subcommittee of the Scientific Advisory Committee. Edinburgh: H.M. Stationery Office.
(383) Macdonald (1947). *Dairy Industr.* **12**, 846.
(384) Aschaffenburg & Temple (1941). *J. Dairy Res.* **12**, 315.
(385) Aschaffenburg & Veinoglou (1944). *J. Dairy Res.* **13**, 267.
(386) Aschaffenburg, Temple & Veinoglou (1944). *J. Dairy Res.* **13**, 281.
(387) Tankard & Bagnall (1944). *Analyst*, **69**, 209.
(388) Lighthelm (1944). *Fmg S. Afr.* **19**, 424.
(389) Koenig (1941). *Z. Untersuch. Lebensmitt.* **81**, 481.
(390) Veinoglou (1945). *Nature, Lond.*, **155**, 172.
(391) Dodd & Cowan (1943). *Analyst*, **68**, 146.
(392) Rowland, Aschaffenburg & Veinoglou (1943). *Analyst*, **68**, 302.
(393) O'Loughlin & Ryan (1944). *J. Dep. Agric. Eire*, **41**, 5.
(394) Monk (1942). *Brit. Food J.* **44**, (519), 11.
(395) Macdonald (1943). *Analyst*, **68**, 171.
(396) Ryan (1944). *Sci. Proc. R. Dublin Soc.* **23** (n.s.), 271.
(397) Kothavalla, Bhalla & Lavangia (1942). *Indian J. vet. Sci.* **12**, 1.
(398) Gutierrez (1941). *Acta med. Philipp.* **2**, 497.
(399) Lythgoe (1945). *J. Milk. Tech.* **8**, 101.
(400) Seixas (1942). *Rev. Inst. Adolfo Lutz*, **2**, 390.
(401) Gross (1941). *Arch. Hyg.* **126**, 219.
(402) De Vleeschauwer & Waeyenberge (1941). *Meded. LandbHoogisch. Opzoek. Gent*, **9**, (1), 56.
(403) Rangappa (1947). *Proc. Indian Acad. Sci.* **25**, (4), B 86.
(404) Mokrants (1938). *Bull. Soc. chim. Yougoslavie*, **9**, 105.
(405) Montefredine (1942). *Ann. Chim. appl., Roma*, **32**, 62.
(406) Occhialini (1940). *Quad. Nutriz.* **7**, 246.
(407) Aschaffenburg (1943). *Nature, Lond.*, **151**, 169.
(408) Rowlson & Mickle (1945). *J. Milk Tech.* **8**, 315.
(409) Moir (1944). *Analyst*, **69**, 17.
(410) King & Schouest (1944). *Quart. Bull. Ass. Food Drug Officials U.S.* **8**, 136.
(411) (1946). *Indian Fmg*, **7**, 416.
(412) Liebenthal & Adolph (1941). *Chin. med. J.* **60**, 174.
(413) Gutierrez & Sunico-Suaco (1941). *Acta med. Philipp.* **2**, 351.
(414) Smith (1942). *J. Pediat.* **20**, 616.
(415) Zaribnicky (1942). *Z. Kinderheilk.* **63**, 543.
(416) Rodkey & Ball (1946). *J. Lab. clin. Med.* **31**, 354.

(417) (1943). *Indian Fmg*, **4**, 205.
(418) (1944). *Indian Fmg*, **5**, 81.
(419) Dastur, Kashyap & Kothavalla (1944). *Indian J. vet. Sci.* **14**, 49.
(420) Dastur, Kashyap & Kothavalla (1944). *Indian J. vet. Sci.* **14**, 58.
(421) Narayanaier (1945). *Curr. Sci.* **14**, 177.
(422) Achaya & Banerjee (1945). *Indian J. vet. Sci.* **15**, 261.
(423) Krienke (1944). *J. Dairy Sci.* **27**, 683.
(424) Corbett & Tracy (1943). *J. Dairy Sci.* **26**, 1095.
(425) Freeman (1942). *Milk Dlr*, **32**, (3), 26.
(426) Josephson, Burgwald & Stoltz (1946). *J. Dairy Sci.* **29**, 273.
(427) Josephson (1946). *J. Dairy Sci.* **29**, 508.
(428) Danielsson (1947). *Medd. Svenska Mejeriern. Riksfören.* no. 2.
(429) England & Wiedemer (1941). *Milk Dlr*, **31**, (3), 33.
(430) Trout, Bryan & Horwood (1942). *Quart. Bull. Mich. agric. Exp. Sta.* **24**, 342.
(431) Keeney & Josephson (1947). *J. Dairy Sci.* **30**, 535.
(432) Saal & Heukelom (1946). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 52 (3) G; English version in : *Monographs on the Progress of Research in Holland during the War*, no. 12. New York and Amsterdam: Elsevier Publ. Co. Inc. (1947).
(433) Hartman, Garrett & Button (1943). *J. Dairy Sci.* **26**, 515.
(434) Corbett & Tracy (1943). *J. Dairy Sci.* **26**, 419.
(435) Horrall & Epple (1941). *Rep. Ind. agric. Exp. Sta.* 1940–1, p. 56.
(436) Epple & Horrall (1943). *J. Dairy Sci.* **26**, 525.
(437) Krukovsky & Guthrie (1945). *J. Dairy Sci.* **28**, 565.
(438) Krukovsky & Guthrie (1946). *J. Dairy Sci.* **29**, 293.
(439) Guthrie (1946). *J. Dairy Sci.* **29**, 359.
(440) Leeder & Herreid (1942). *Bull. Vt agric. Exp. Sta.* no. 481.
(441) Hartman & Garrett (1943). *J. Dairy Sci.* **26**, 337.
(442) Olson & Brown (1942). *J. Dairy Sci.* **25**, 1027, 1041.
(443) Olson & Brown (1944). *J. Dairy Sci.* **27**, 197, 205.
(444) Hartmann (1944). *Beretn. Forsøgsm., Kbh.*, no. 41.
(445) Castell (1942). *J. Milk Tech.* **5**, 195.
(446) Russell & Dahle (1943). *J. Dairy Sci.* **26**, 25.
(447) Tarassuk & Henderson (1943). *J. Dairy Sci.* **26**, 774.
(448) Tarassuk & Henderson (1946). *J. Dairy Sci.* **29**, 501.
(449) Mueller & Mack (1940). *Rep. Mass. agric. Exp. Sta.* 1938–9, (*Bull.* no. 369), 42.
(450) Mueller & Mack (1941). *Rep. Mass. agric. Exp. Sta.* 1939–40, (*Bull.* no. 378), 51.
(451) Stevens. Brit. Pat. 546,593, 13. xi. 40.
(452) Nelson (1943). *Bull. Mont. agric. Exp. Sta.* no. 410.
(453) Patton & Josephson (1947). *J. Dairy Sci.* **30**, 538.
(454) Hankinson, Ellertson & Gleim (1946). *J. Dairy Sci.* **29**, 727.
(455) Josephson & Keeney (1947). *Milk Dlr*, **36**, (10), 40.
(456) Gould (1944). *J. Dairy Sci.* **27**, 167.
(457) Moore & Trout (1946). *Canad. Dairy Ice Cr. J.* **25**, (7), 33.
(458) Barnhart (1940). *Abstr. Doc. Dissert. Pa St. Coll.* **3**, 215.
(459) Stull, Herreid & Tracy (1947). *J. Dairy Sci.* **30**, 541.
(460) Van der Waarden (1944). *Versl. landb. Onderzoek. Dep. Landb., 's Grav.*, no. 50 (2) G, 21. English version in : *Monographs on the Progress of Research in Holland during the War*, no. 12. New York and Amsterdam: Elsevier Publ. Co. Inc. (1947).
(461) Van der Waarden (1945). *Versl. algem. ned. Zuivelb., 's Grav.*, 1943–4, p. 6.
(462) Davies (1941). *J. Indian chem. Soc.* (Industr. ed.), **4**, 1, 150, 175, 179.
(463) Krienke & Fouts (1942). *Proc. Ass. Sth. agric. Wkrs*, 1942, p. 121.
(464) Hussong & Hammer (1944). *Food Res.* **9**, 289.
(465) Hussong, Quam & Hammer (1944). *J. Dairy Sci.* **27**, 45.
(466) Bird & Fabricius (1941–2). *Rep. Ia agric. Exp. Sta.* 1940–1, I, 149; 1941–2, I, 167.
(467) Aas & Borgen (1941). *Meieriposten*, **30**, 357, 375.

(468) JØRGENSEN (1944). *Aarsberetn. St. Smørbedømm.* 1943, p. 56.
(469) SWARTLING (1947). *Svenska Mejeritidn.* **39**, 399.
(470) OLSON (1947). *Abstr. Proc. S. Div. Amer. Dairy Sci. Ass.* 1947, p. 21.
(471) MCDOWALL, MORTON & MCDOWELL (1947). *N.Z. J. Sci. Tech.* A, **28**, 305.
(472) MCDOWALL, MORTON, O'DEA & ALLO (1947). *N.Z. J. Sci. Tech.* A, **28**, 370.
(473) HANSEN & THEOPHILUS (1942). *Rep. Idaho agric. Exp. Sta.* 1941, (*Bull.* no. 244).
(474) WHITE & GIBSON (1946). *Canad. Dairy Ice. Cr. J.* **25**, (2), 35; (3), 39.
(475) SABALITSCHKA (1940). *Z. Untersuch. Lebensmitt.* **79**, 143.
(476) GLIMM & NOWACK (1943). *Fette u. Seifen*, **50**, 217.
(477) KAUFMANN & WOLF (1943). *Fette u. Seifen*, **50**, 218.
(478) GOULD (1946). *Nat. Butt. Cheese J.* **37**, (1), 40.
(479) DAVIES (1940). *J. Indian chem. Soc.* (Industr. ed.), **3**, 174.
(480) EL-RAFEY, RICHARDSON & HENDERSON (1944). *J. Dairy Sci.* **27**, 807.
(481) HOLM, SCHAFFER & HALLER (1943). *J. Dairy Sci.* **26**, 760.
(482) GLIMM & ELLERT (1941). *Fette u. Seifen*, **48**, 60.
(483) SOLNTSEV (1940). *Zh. prikl. Khim., Mosk.* **13**, 1389.
(484) LEA (1944). *J. Soc. chem. Ind., Lond.*, **63**, 107.
(485) HINKO (1942). *Fette u. Seifen*, **49**, 19.
(486) LOFTUS-HILLS & CONOCHIE (1945). *J. Coun. sci. industr. Res. Aust.* **18**, 355.
(487) LOFTUS-HILLS & CONOCHIE (1946). *J. Coun. sci. industr. Res. Aust.* **19**, 414.
(488) ACHAYA (1947). *Nature, Lond.*, **159**, 274.
(489) DAHLE & JOSEPHSON (1943). *Nat. Butt. Cheese J.* **34**, (10), 18.
(490) SCHAFFER, GREENBANK & HOLM (1946). *J. Dairy Sci.* **29**, 145.
(491) COULTER & JENNESS (1947). *J. Dairy Sci.* **30**, 528.
(492) REMALEY (1946). *Nat. Butt. Cheese J.* **37**, (3), 36.
(493) WILLIAMSON (1944). *Food Res.* **9**, 298.
(494) HOLLENDER & TRACY (1942). *J. Dairy Sci.* **25**, 249.
(495) TRACY & HOSKISSON (1944). *J. Dairy Sci.* **27**, 311.
(496) CHAPMAN & MCFARLANE (1946). *Canad. J. Res.* **24**, Sect. F, 47.
(497) FINDLAY, SMITH & LEA (1945). *J. Dairy Res.* **14**, 165.
(498) HLYNKA, HOOD & GIBSON (1943). *J. Dairy Sci.* **26**, 1111.
(499) HLYNKA, HOOD & GIBSON (1945). *J. Dairy Sci.* **28**, 79.
(500) HLYNKA & HOOD (1947). *J. Dairy Res.* **15**, 94.
(501) HLYNKA, HOOD & GIBSON (1947). *Sci. Agric.* **27**, (1), 50.
(502) CHILLINGWORTH & GILLESPIE (1946). *Aust. J. Dairy Tech.* **1**, 127.
(503) WHITMAN, SLACK & HERREID (1943). *Bull. Vt agric. Exp. Sta.* no. 502.
(504) HELLSTRAND (1942). *Svenska Mejeritidn.* **34**, 53.
(505) ANDERSEN (1946). *Aarsberetn. Forsøgsm., Kbh.*, 1945–6, Beretn. no. 52, 23.
(506) SCHLOEMER, RAUCH & SCHLOEMER (1942). *Z. Untersuch. Lebensmitt.* **83**, 305.
(507) HERREID (1942). *J. Dairy Sci.* **25**, 335.
(508) HERRINGTON (1944). *J. Dairy Sci.* **27**, 857.
(509) JENNESS, HERREID, CAULFIELD, BURGWALD, JACK & TUCKEY (1942). *J. Dairy Sci.* **25**, 949.
(510) MARTIN, CAULFIELD, BECK, NELSON, FOLTZ, WHITNAH & PARSONS (1940). *Bienn. Rep. Kans. agric. Exp. Sta.* 1938–40, p. 87.
(511) LAMPERT (1943). *Mon. Bull. Calif. Dep. Agric.* **32**, 83.
(512) HERREID, JENNESS & WHITMAN (1943). *J. Dairy Sci.* **26**, 883.
(513) JENNESS & HERREID (1945). *J. Dairy Sci.* **28**, 591.
(514) HERREID & HARMON (1944). *J. Dairy Sci.* **27**, 33.
(515) HERRINGTON (1944). *J. Dairy Sci.* **27**, 67.
(516) HERREID & WHITMAN (1944). *J. Dairy Sci.* **27**, 147.
(517) HILEMAN, RUSH & MOSS (1942). *J. Dairy Sci.* **25**, 373.
(518) ELLENBERGER (1941). *Bull. Vt agric. Exp. Sta.* no. 475, 23.
(519) HERREID, WHITMAN & SLACK (1944). *Bull. Vt agric. Exp. Sta.* no. 512.
(520) FILL & STOCK (1945). *Analyst*, **70**, 22.
(521) CADOR & MACHEREL (1945). *Lait*, **25**, 289.

(522) Ram Ayyar & Kothavalla (1943). *Indian J. vet. Sci.* **13**, 133.
(523) Janse (1946). *Chem. Weekbl.* **42**, 284.
(524) Schloemer & Rauch (1942). *Z. Untersuch. Lebensmitt.* **83**, 289.
(525) Reid (1945). *Dairy Industr.* **10**, 561.
(526) Trout & Lucas (1947). *J. Dairy Sci.* **30**, 145.
(527) (1942). *Rep. Ariz. agric. Exp. Sta.* 1940–1, p. 54.
(528) Harland & Davis (1946). *J. Dairy Sci.* **29**, 845.
(529) Trout & Lucas (1945). *J. Dairy Sci.* **28**, 901.
(530) Lucas & Trout (1947). *J. Dairy Sci.* **30**, 95.
(531) Marquardt (1942). *Milk Dlr*, **31**, (5), 24.
(532) Hansen & Deane (1940). *Proc. W. Div. Amer. Dairy Sci. Ass.* 1940, p. 31.
(533) Hansen, Horrall, Bird & Jensen (1945). *J. Dairy Sci.* **28**, 325.
(534) Hostettler, Lehmann & Künzle (1946). *Mitt. Lebensm. Hyg., Bern*, **37**, 238.
(535) Mohr & Baur (1941). *Fette u. Seifen*, **48**, (8), 8.
(536) Sandelin (1945). *Meijerit. Aikakausk.* **7**, 104.
(537) Mulder (1943). *Ned. Weekbl. Zuivelbereiding en-Handel*, 1943, (20), 1.
(538) (1944). *J. Ass. off. agric. Chem., Wash.*, **27**, 337.
(539) Mohr & Pasveer (1942). *Dtsch. Molkereiztg*, **63**, 602.
(540) Schwarz & Mumm (1943). *Dtsch. Molkerei- u. Fettw.* **1**, 349.
(541) Heinemann (1946). *J. Dairy Sci.* **29**, 689.
(542) Hurel & Mocquot (1946). *Lait*, **26**, 289.
(543) Sandelin (1941). *Meijerit. Aikakausk.* **3**, 104.
(544) Mulder (1947). *Off. Org. algem. ned. Zuivelb.* **39**, (14), 108.
(545) Mulder (1940). *Versl. landb. Onderzoek. Dep. Landb., 's Grav.*, no. 46 (13) C, 505.
(546) Adriani & Tamsma (1945). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 51 (6) G, 79.
(547) Johnson & Gould (1946). *J. Dairy Sci.* **29**, 504.
(548) (1945). *Analyst*, **70**, 105.
(549) Livak (1943). *Abstr. Doc. Dissert. Pa St. Coll.* **6**, 94.
(550) Doan & Livak (1945). *J. Dairy Sci.* **28**, 921.
(551) Stuart & Martin (1942). *Dairy Industr.* **7**, 281.
(552) Bakalor (1946). *Fmg S. Afr.* **21**, 531.
(553) Bohm (1940). *Z. Untersuch. Lebensmitt.* **80**, 241.
(554) Boden & Campbell (1942). *J. Dairy Res.* **13**, 45.
(555) Desai & Patel (1945). *Curr. Sci.* **14**, 203.
(556) Rueda (1943). *Nat. Butt. Cheese J.* **34**, (8), 12.
(557) Herrington (1946). *J. Dairy Sci.* **29**, 87.
(558) Livak (1944). *Nat. Butt. Cheese J.* **35**, (1), 13.
(559) Schneck & Plottke (1940). *Vorratspfl. u. Lebensmittelforsch.* **3**, 349.
(560) Rangappa (1947). *Nature, Lond.*, **160**, 719.
(561) Alcock (1946). *Analyst*, **71**, 233.
(562) Sarudi (1941). *Z. Untersuch. Lebensmitt.* **82**, 451.
(563) Jonnard (1945). *Industr. Engng Chem.* (Anal. ed.), **17**, 246.
(564) Miller & Houghton (1945). *J. biol. Chem.* **159**, 373.
(565) Ling (1942). *J. Soc. chem. Ind., Lond.*, **61**, 194.
(566) Münchberg & Sulinskas (1941). *Milchw. Forsch.* **21**, 93.
(567) Choi, O'Malley & Fairbanks (1946). *J. Dairy Sci.* **29**, 645.
(568) Thomé (1942). *Medd. Statens Mejeriförsök*, no. 9.
(569) Thomé (1946). *Medd. Statens Mejeriförsök*, no. 18, 150.
(570) Overby & Madsen (1947). *Maelkeritidende*, **60**, (1), 7.
(571) Brereton & Sharp (1942). *Industr. Engng Chem.* (Anal. ed.), **14**, 872.
(572) Wokes & Still (1942). *Biochem. J.* **36**, 797.
(573) Doherty & Ogg (1943). *Industr. Engng Chem.* (Anal. ed.), **15**, 759.
(574) F[illegible]nkel-Conrat & Cooper (1944). *J. biol. Chem.* **154**, 239.
(575) Vi[illegible]nen & Rautanen (1947). *Biochem. J.* **41**, 101.
(576) Hauschildt, Isaacs & Wallace (1947). *J. biol. Chem.* **167**, 331.

(577) DE VLEESCHAUWER & HEYNDRICKX (1946). *Meded. LandbHoogesch. Opzoek. Gent*, **11**, 304.
(578) HORVATH (1946). *Industr. Engng Chem.* (Anal. ed.), **18**, 229.
(579) NEUBERG, STRAUSS & LIPKIN (1944). *Arch. Biochem.* **4**, **101**.
(580) PYNE (1943). *Analyst*, **68**, 330.
(581) WUHRMANN & HÖGL (1944). *Mitt. Lebensm. Hyg., Bern*, **35**, 273.
(582) VLADESCO (1941). *C.R. Acad. Sci., Paris*, **212**, 394.
(583) VLADESCO (1941). *C.R. Acad. Sci., Paris*, **212**, 641.
(584) VAN SLYKE & HILLER (1947). *J. biol. Chem.* **167**, 107.
(585) GROSSFELD & YOUNG YEN (1941). *Z. Untersuch. Lebensmitt.* **82**, 489.
(586) MCDOWELL (1947). *J. Dairy Res.* **15**, 70.
(587) DEATH, LIGHTFOOT & MOIR (1946). *N.Z. J. Sci. Tech.* **28**, A, 273.
(588) CRANSTON & THOMPSON (1946). *Industr. Engng Chem.* (Anal. ed.), **18**, 323.
(589) WOIWOD (1947). *Biochem. J.* **41**, 39.
(590) ELLIS & THOMPSON (1945). *Industr. Engng Chem.* (Anal. ed.), **17**, 254.
(591) OWEN (1946). *Analyst*, **71**, 210.
(592) BONOLDI (1942). *Rev. Fac. Med. vet. S. Paulo*, **2**, (2), 13.
(593) (1944). *Analyst*, **69**, 243.
(594) MÜHLSCHLEGEL (1940). *Z. Untersuch. Lebensmitt.* **80**, 70.
(595) GARRISON & HAIGH (1942). *J. Ass. off. agric. Chem., Wash.*, **25**, 603.
(596) RAUSCHNING (1942). *Milchw. Forsch.* **21**, 249.
(597) PELTZER (1941). *Chemikerztg*, **65**, 331.
(598) HEINEMANN (1944). *J. Dairy Sci.* **27**, 377.
(599) EILERS & JENSE (1945). *Versl. landb. Onderzoek., Minist. Landb., 's Grav.*, no. 50 (15) G, 1081.
(600) ZILLIOX, MITCHELL & FRARY (1947). *J. Ass. off. agric. Chem., Wash.*, **30**, 130.
(601) HILLIG (1943). *J. Ass. off. agric. Chem., Wash.*, **26**, 199.
(602) HILLIG (1944). *J. Ass. off. agric. Chem., Wash.*, **27**, 235.
(603) GOULD (1944). *J. Dairy Sci.* **27**, 743.
(604) GOULD (1945). *Quart. Bull. Mich. agric. Exp. Sta.* **28**, 115.
(605) GOULD & JENSEN (1944). *J. Dairy Sci.* **27**, 753.
(606) ZARINS & BRANTS (1943). *Z. Lebensmitt.* **86**, 19.
(607) GROSSFELD & ZEISSET (1943). *Z. Untersuch. Lebensmitt.* **85**, 321.
(608) TOWNLEY & GOULD (1943). *J. Dairy Sci.* **26**, 689.
(609) DIEMAIR, STROHECKER & KELLER (1939). *Vorratspfl. u. Lebensmittelforsch.* **2**, 615.
(610) HARTMAN & GARRETT (1942). *Industr. Engng Chem.* (Anal. ed.), **14**, 641.
(611) HORN (1944) *Publ. Wagner Free Inst. Sci., Philadelphia*, **4**, **1**.
(612) SCHECHTER, POGORELSKIN & HALLER (1947). *Analyt. Chem.* [*Industr. Engng Chem.* (Anal. ed.)], **19**, 51.
(613) CARTER (1947). *Analyt. Chem.* [*Industr. Engng Chem.* (Anal. ed.)], **19**, 54.
(614) WILSON (1946). *J. Ass. off. agric. Chem., Wash.*, **29**, 311.
(615) BÄCKSTRÖM (1942). *Z. anal. Chem.* **123**, 96.
(616) SCHWARZ & HAGEMANN (1941). *Vorratspfl. u. Lebensmittelforsch.* **4**, 50.
(617) PEDERSEN (1944). *Aarsberetn. Forsogsm., Kbh.*, 1943–4, Beretn. no. 43, 18.
(618) PIEN (1946/47). *Industr. Lait*, no. 3/4, new ser., 10.
(619) MACDONALD (1944). *Analyst*, **69**, 173.
(620) CLARKE (1947). *Amer. J. publ. Hlth*, **37**, 728.
(621) RUDNICK (1947). *Amer. Butt. Rev.* **9**, 22.
(622) MENNICKE (1942). *Milchw. Forsch.* **21**, 252.
(623) MENNICKE (1943). *Milchw. Forsch.* **21**, 261.
(624) MOHR & EYSANK (1943). *Fette u. Seifen*, **50**, 145.
(625) LOFTUS-HILLS & CONOCHIE (1945). *J. Coun. sci. industr. Res. Aust.* **18**, 366.
(626) MORELL, CONOCHIE & LOFTUS-HILLS (1946). *J. Coun. sci. industr. Res. Aust.* **19**, 190.
(627) SABALITSCHKA (1941). *Ber. dtsch. chem. Ges.* **74**, Abt. B, 1040.
(628) PARMELEE, BIRD & BREAZEALE (1943). *Res. Bull. Ia agric. Exp. Sta.* no. 316, 656.
(629) WHITE (1946). *Canad. Dairy Ice Cr. J.* **25**, (8), 23.
(630) VORHES (1946). *J. Ass. off. agric. Chem., Wash.*, **29**, 119.
(631) MEURON (1946). *J. Ass. off. agric. Chem., Wash.*, **29**, 125.

(632) Ritter (1946). *Schweiz. Milchztg*, **72**, Nos. (11) and (12).
(633) Götel & Effern (1940). *Vorratspfl. u. Lebensmittelforsch.* **3**, 158.
(634) Mohr & Baur (1941). *Molkereiztg, Hildesh.*, **55**, (3), 31.
(635) Olsson (1941). *Svenska Mejeritidn.* 1941, no. 31.
(636) Andersen (1946). *Aarsberetn. Forsøgsm., Kbh.*, 1945–6, Beretn. no. 52, 74.
(637) Heinemann (1945). *J. Dairy Sci.* **28**, 845.
(638) Thiel (1943). *J. Coun. sci. industr. Res. Aust.* **16**, 135.
(639) Korpáczy & Ersek (1942). *Z. Untersuch. Lebensmitt.* **83**, 218.
(640) Nussbaumer & Ritter (1944). *Schweiz. Milchztg*, **70**, 117.
(641) Mohr & Mack (1942). *Dtsch. Molkereiztg*, **63**, 546.
(642) King (1947). *Neth. Milk Dairy J.* **1**, 19.
(643) Mohr & Eysank (1939). *Vorratspfl. u. Lebensmittelforsch.* **2**, 621.
(644) Kaufmann & Lund (1940). *Fette u. Seifen.* **47**, 570.
(645) Barnicoat (1944). *Analyst*, **69**, 176.
(646) Kruisheer, den Herder, Krol & Ginkel, van (1943). *Handb. Genoot. Melkkunde*, 1943.
(647) (1943). *Versl. Rijkszuivst., Leiden*, 1942, p. 12.
(648) Brouwer (1943). *Ned. Weekbl. Zuivelbereiding en-Handel*, **49**, 24 Aug.
(649) Grant & White (1946). *Canad. J. Res.* **24**, F, 461.
(650) Effern (1939). *Vorratspfl. u. Lebensmittelforsch.* **2**, 672.
(651) Kosikowsky, Dahlberg, Guthrie & Knaysi (1947). *J. Dairy Sci.* **30**, 211.
(652) Schloemer (1940). *Z. Untersuch. Lebensmitt.* **80**, 329.
(653) Støren & Dovle (1944). *Meld. Norg. LandbrHøgsk. MeiriAvd.* no. 22.
(654) Knaysi (1942). *J. Dairy Sci.* **25**, 585.
(655) Krukovsky & Knaysi (1942). *J. Dairy Sci.* **25**, 659.
(656) Knaysi & Guthrie (1942). *J. Dairy Sci.* **25**, 589.
(657) Rieman (1943). *Industr. Engng Chem.* (Anal. ed.), **15**, 325.
(658) Bird (1941/42). *Rep. Ia agric. Exp. Sta.* 1940–1, I, 142; 1941–2, I, 162.
(659) Mulder (1940). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 46 (11) C, 439.
(660) Platon & Olsson (1941). *Medd. Statens Mejeriförsök*, no. 8.
(661) Aas (1943). *Meieriposten*, **32**, 37.
(662) Janse (1944). *Landb. Tijdschr. ned. Genoot. LandbWetensch.* **56**, 28.
(663) Lea (1946). *J. Soc. chem. Ind., Lond.*, **65**, 286.
(664) Mummery (1947). *J. Dairy Res.* **15**, 55.
(665) Mangold (1941). *Vorratspfl. u. Lebensmittelforsch.* **4**, 297.
(666) Sundberg & Hultberg (1942). *Iva*, **13**, 342.
(667) Sylvester, Lampitt & Ainsworth (1942). *J. Soc. chem. Ind., Lond.*, **61**, 165.
(668) Beier (1939). *Vorratspfl. u. Lebensmittelforsch.* **2**, 201.
(669) Kiermeier (1940). *Fette u. Seifen*, **47**, 564.
(670) Brouwer (1944). *Handb. Genoot. Melkkunde*, 1944, I.
(671) Brouwer (1946). *Acta brev. neerl. Physiol.* **13**, 74.
(672) Schloemer (1940). *Z. Untersuch. Lebensmitt.* **79**, 463.
(673) Voorst, van (1947). *Chem. Weekbl.* **43**, (4), 54.
(674) Henderson & Jack (1944). *Oil & Soap*, **21**, 90.
(675) Hamdi & Zollikofer (1944). *Mitt. Lebensm. Hyg., Bern*, **35**, 66.
(676) Gould & Johnson (1944). *J. Dairy Sci.* **27**, 173.
(677) Grossfeld (1940). *Z. Untersuch. Lebensmitt.* **80**, 434.
(678) Ramsey & Patterson (1945). *J. Ass. off. agric. Chem., Wash.*, **28**, 644.
(679) Bohm, Sturz & Jonas (1943). *Z. Untersuch. Lebensmitt.* **85**, 334.
(680) Arbuckle (1946). *J. Dairy Sci.* **29**, 431.
(681) Vilegzhanin (1942). *Proc. Vologda agric. Inst.* 1942, v, 311.
(682) Horrall & Crane (1940). *Rep. Ind. agric. Exp. Sta.* 1939–40, p. 48.
(683) Mulder (1942). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 48 (12) C, 709.
(684) Crane & Horrall (1942). *J. Dairy Sci.* **25**, 651.
(685) Sirks (1939). *Versl. landb. Onderzoek., Dep. Landb., 's Grav.*, no. 45 C, 575.
(686) Schwarz, Fischer & Hagemann (1943). *Dtsch. Molkereiztg*, **64**, 143.

(687) (1946). *Misc. Bull. Imp. Coun. agric. Res.* no. 64.
(688) MULDER (1940). *Off. Org. algem. ned. Zuivelb.* 1940, no. 38.
(689) RITTER (1941). *Schweiz. Milchztg*, **67**, (26), 116.
(690) EREKSON (1946). *Nat. Butt. Cheese J.* **37**, (8), 46.
(691) HOOD & SPROULE (1944). *Canad. Dairy Ice Cr. J.* **23**, (6), 27.
(692) THIBODEAU (1947). *Canad. Dairy Ice Cr. J.* **26**, (10), 31.
(693) TURNER, ROGERS & CONQUEST (1942). *J. Dairy Sci.* **25**, 777.
(694) MIERSCH & PRICE (1944). *J. Dairy Sci.* **27**, 881.
(695) JULIEN, DUMAIS & THIBODEAU (1947). *Canad. Dairy Ice Cr. J.* **26**, (10), 34.
(696) O'SHEA & LYONS (1941). *Econ. Proc. R. Dublin Soc.* **3**, 91.
(697) GOULD (1942). *Quart. Bull. Mich. agric. Exp. Sta.* **24**, 318.
(698) GROSSFELD & ZEISSET (1942). *Z. Untersuch. Lebensmitt.* **84**, 193.
(699) BABEL & NELSON (1944). *Nat. Butt. Cheese J.* **35**, (9), 18.
(700) ZEISSET (1942). *Z. Untersuch. Lebensmitt.* **84**, 301.
(701) GARARD (1942). *J. Ass. off. agric. Chem., Wash.*, **25**, 594.
(702) SMILEY, KOSIKOWSKY & DAHLBERG (1946). *J. Dairy Sci.* **29**, 307.
(703) KOSIKOWSKY & DAHLBERG (1946). *J. Dairy Sci.* **29**, 861.
(704) DAHLBERG & KOSIKOWSKY (1947). *J. Dairy Sci.* **30**, 165.
(705) KOESTLER (1943). *Annu. agric. Suisse*, **44**, 499.
(706) SCHWARZ & MUMM (1942). *Molkereiztg, Hildesh.*, **56**, 459.
(707) ANDERSEN (1946). *Aarsberetn. Forsøgsm., Kbh.*, 1944–5, Beretn., no. 50, 21.
(708) SANDELIN (1941). *Meijerit. Aikakausk.* **3**, 48.
(709) ARBUCKLE (1946). *Nat. Butt. Cheese J.* **37**, (5), 41.
(710) SCHWARZ & FISCHER (1941). *Käse-Industr., Hildesh.*, **4**, 33.
(711) SEUSS (1940). *Vorratspfl. u. Lebensmittelforsch.* **3**, 410.
(712) EFFERN (1941). *Vorratspfl. u. Lebensmittelforsch.* **4**, 58.
(713) CSISZÁR & BAKOS (1941). *Milchw. Forsch.* **21**, 56.
(714) OLSSON (1943). *Svenska Mejeritidn.* **35**, no. 6.
(715) LAWRENCE (1946). *Aust. J. Dairy Tech.* **1**, 48.
(716) CONE & ASHWORTH (1947). *J. Dairy Sci.* **30**, 463.
(717) CHAPMAN & MCFARLANE (1943). *Canad. J. Res.* **21**, B, 133.
(718) LEA (1945). *J. Soc. chem. Ind., Lond.*, **64**, 106.
(719) LOFTUS-HILLS & THIEL (1946). *J. Dairy Res.* **14**, 340.
(720) LOFTUS-HILLS & WILKINSON (1946). *J. Coun. sci. industr. Res. Aust.* **19**, 430.
(721) JENNESS, COULTER & LARSON (1946). *J. Dairy Sci.* **29**, 491.
(722) PYENSON & TRACY (1946). *J. Dairy Sci.* **29**, 1.
(723) CHAPMAN & MCFARLANE (1945). *Canad. J. Res.* **23**, B, 91.
(724) HARLAND & ASHWORTH (1945). *J. Dairy Sci.* **28**, 15.
(725) MUERS & ANDERSON (1944). *Analyst*, **69**, 5.
(726) LEA & GANE (1946). *J. Dairy Res.* **14**, 400.
(727) CARTWRIGHT (1946). *Industr. Engng Chem.* (Anal. ed.), **18**, 779.
(728) GROSSFELD & SCHROEDER (1943). *Z. Untersuch. Lebensmitt.* **85**, 225.
(729) TERRIER (1941). *Mitt. Lebensm. Hyg., Bern*, **32**, 237.
(730) SCHLOEMER & CATRAVAS (1940). *Z. Untersuch. Lebensmitt.* **79**, 459.
(731) SCHWARZ & HAGEMANN (1941). *Dtsch. Molkereiztg*, **62**, 554.
(732) SCHWARZ & MUMM (1942). *Dtsch. Molkereiztg*, **63**, 229.
(733) HILLIG (1942). *J. Ass. off. agric. Chem., Wash.*, **25**, 610.
(734) HILLIG (1945). *J. Ass. off. agric. Chem., Wash.*, **28**, 205.
(735) KUNKEL & COMBS (1943). *J. Dairy Sci.* **26**, 769.
(736) KUNKEL & COMBS (1945). *J. Dairy Sci.* **28**, 219.
(737) COULTER & KUNKEL (1944). *J. Dairy Sci.* **27**, 671.
(738) HILLIG (1942). *J. Ass. off. agric. Chem., Wash.*, **25**, 602.
(739) STAMBERG & BOLIN (1947). *Analyt. Chem.* [*Industr. Engng Chem.* (Anal. ed.)], **19**, 695.
(740) MENEFEE (1945). *J. Dairy Sci.* **28**, 243.
(741) HETRICK & TRACY (1945). *J. Milk Tech.* **8**, 5.

(742) Boulet & McFarlane (1945). *Canad. J. Res.* **23**, B, 70.
(743) Morrison & Paige (1946). *Industr. Engng Chem.* (Anal. ed.), **18**, 211.
(744) Thompson (1944). *Industr. Engng Chem.* (Anal. ed.), **16**, 646.
(745) Pyenson & Tracy (1945). *J. Dairy Sci.* **28**, 401.
(746) Choi, O'Malley & Fairbanks (1946). *J. Dairy Sci.* **29**, 639.
(747) Brown & Thurston (1943). *J. Milk Tech.* **6**, 136.
(748) Eastman & Maybee (1947). *Canad. Dairy Ice Cr. J.* **26**, (5), 64.
(749) Brown, Herreid, Erb & Swope (1944). *J. Dairy Sci.* **27**, 53.
(750) Stanworth (1944). *Dairy Industr.* **9**, 702.
(751) Miller, Zimmerman & Oberg (1947). *J. Dairy Sci.* **30**, 531.
(752) Leighton (1942–7). *Ice Cream Rev.* (1942), **25**, (9); (1943), **26**, (9), 18; (1944), **27**, (9) 26; (1945), **28**, (10), 38; (1946), **29**, (11), 41; (1947), **30**, (11), 45.

REFERENCES ARRANGED ALPHABETICALLY

Aas (134, 661); Aas & Borgen (467); Acharya & Devadatta (65); Achaya (488); Achaya & Banerjee (93, 94, 95, 422); Adriani & Tamsma (373, 546); Adriani *et al.* (41); Alcock (561); Amaral Rogick (12); Anantakrishnan & Kothavalla (66); Anantakrishnan & Lahiry (114); Anantakrishnan *et al.* (37, 67, 92); Andersen, A. C. (186); Andersen, A. C. & Vestesen (182); Andersen, K. & Hartmann (180); Andersen, K. P. (505, 636, 707); Angelescu (47); Araujo (237); Arbuckle (680, 709); Archibald (151); Aschaffenburg (185, 307, 407); Aschaffenburg & Temple (384); Aschaffenburg & Veinoglou (385); Aschaffenburg *et al.* (386); Ashworth & Van Orden (62).

Babcock (327); Babcock *et al.* (353, 354); Babel & Hammer (221); Babel & Nelson (699); Bäckström (615); Bakalor (8, 552); Baldwin & Longenecker (68, 89); Barber & Frazier (189); Barnhart (458); Barnicoat (645); Basu & Mukherjee (141); Beier (668); Bell & Webb (351); Bergdoll & Doty (107); Berridge (197, 198); Bever, van (165); Bever, van & Straub (166); Bird (372, 658); Bird & Fabricius (466); Boden & Campbell (554); Bohm (553); Bohm *et al.* (679); Bolling & Block (102); Bonnier *et al.* (33); Bonoldi (592); Boulet & McFarlane (742); Brand & Kassell (281, 282); Brasch & Huber (339); Braude *et al.* (39); Briggs & Hanig (290); Brereton & Sharp (571); Briggs & Hull (296); Brouwer (648, 670, 671); Brouwer & Jonker-Scheffener (70); Brown J. B. & Orians (90); Brown, W. C. & Thurston (747); Brown, W. C. *et al.* (749); Brown, W. H. & Elliker (188); Bull (285, 286, 299); Bull & Currie (284); Burg, van der (249); Burwald (177).

Cador & Macherel (521); Cannan *et al.* (292); Caputto & Marsal (160, 161); Carpenter & Lovelace (276, 277); Carter (613); Cartwright (727); Caserio (46); Castell (445); Caulfield & Martin (191); Chanutin *et al.* (245); Chapman & McFarlane (496, 717, 723); Cheesman & Ehrensvärd (124); Chibnall (99, 100); Chibnall *et al.* (101, 103); Chillingworth & Gillespie (502); Choi *et al.* (567, 746); Clarke (620); Cohen (118); Cole & Tarassuk (127); Cone & Ashworth (716); Corbett & Tracy (424, 434); Coulter & Jenness (360, 491); Coulter & Kunkel (737); Crane & Horrall (154, 684); Cranston & Thompson (588); Csiszár & Bakos (713); Csiszár *et al.* (52).

Dahle & Josephson (489); Dahlberg & Kosikowsky (704); Danielsson (428); Dastur *et al.* (419, 420); Davel (7); Davies (462, 479); Davis & Harland (25); DeAth *et al.* (587); Decker & Reid (133); De Kadt & Minnen (248); De La Mare & Shorland (97); Desai & Patel (555); Deutsch (304); De Vleeschauwer & Heyndrickx (577); De Vleeschauwer & Waeyenberge (402); Diemair & Häusser (229); Diemair *et al.* (609); Dills & Nelson (131); Doan & Livak (550); Doan & Mykleby (334); Doan & Warren (355); Doan *et al.* (332); Dodd & Cowan (391); Doherty & Ogg (573); Doob *et al.* (364); Dunkley & Sommer (315); Dunn & Rockland (111).

Eastman & Maybee (748); Effern (650, 712); Eilers (247); Eilers & Jense (599); Ellenberger (518); Ellis & Thompson (590); El-Rafey & Richardson (308); El-Rafey *et al.* (480); England & Wiedemer (429); Epple & Horrall (436); Erdös (259); Erekson (690); Ewbank & Gould (346).

Fankuchen (287); Farrall *et al.* (337); Feigenbaum (121); Ferraz Lopes & Soares Filho (172); Filipovič (22, 23); Fill & Stock (520); Findlay *et al.* (348, 497); Fischer (175, 226); Fitelson (84); Fraenkel-Conrat & Cooper (574); Fraenkel-Conrat *et al.* (280); Freeman (425); Freeman *et al.* (356); French & Edsall (267); Fritz (376); Frost (119).

Garard (701); Garrett (57); Garrison & Haigh (595); Geyer *et al.* (71); Ghosh & Datta Roy (36); Gilcreas (192); Glimm & Ellert (482); Glimm & Nowack (476); Goodwin & Morton (110); Gordon *et al.* (108); Gorin & Moyer (289); Götel & Effern (633); Gould (75, 217, 218, 340, 456, 478, 603, 604, 697); Gould & Frantz (158); Gould & Jensen (605); Gould & Johnson (676); Gould & Leininger (366); Gould & Skiver (64); Gould *et al.* (368); Grant & White (649); Greenbank *et al.* (358, 362); Grönwall (293, 294, 295, 305); Gross (401); Grossfeld (677); Grossfeld & Schroeder (728); Grossfeld & Young Yen (585); Grossfeld & Zeisset. (607, 698); Guittonneau *et al.* (171, 343); Gutfreund (283); Guthrie (439); Gutierrez (398); Gutierrez & Sunico-Suaco (413).

Hamdi & Zollikofer (675); Hankinson (195, 196); Hankinson & Palmer (199); Hankinson *et al.* (454); Hansen, H. C. & Deane (532); Hansen, H. C. & Theophilus (473); Hansen, H. C. *et al.* (533); Hansen R. G. *et al.* (306); Harland, F. G. (528); Harland, H. A. (130, 724); Harland, H. A. *et al.* (341); Hartman & Garrett (441, 610); Hartman *et al.* (433); Hartmann (181, 444); Hartmann & Andersen (168); Haugaard & Roberts (126); Hauschildt *et al.* (576); Hedrick (87); Hedrick & Hammer (86); Heinemann (27, 63, 541, 598, 637); Hellstrand (504); Henderson & Jack (674); Herman & Peterson (11); Herreid (507); Herreid & Harmon (514); Herreid & Whitman (516); Herreid *et al.* (512, 519); Herrington (508, 515, 557); Herrington & Krukovsky (211); Hershey *et al.* (174); Hetrick & Tracy (361, 741); Hilditch & Jasperson (69, 76, 96); Hilditch & Meara (88, 91); Hileman *et al.* (517); Hillig (601, 602, 733, 734, 738); Hinko (485); Hlynka & Hood (214, 500); Hlynka *et al.* (220, 498, 499, 501); Hoecker & Hammer (60, 85); Holland & Ritchie (146); Hollender & Tracy (494); Holm *et al.* (481); Holmes *et al.* (38, 40); Hood & Sproule (691); Hoover & Kokes (122); Horn (611); Horrall & Crane (682); Horrall & Epple (435); Horvath (578); Horwitz (183, 193); Hostettler (381); Hostettler *et al.* (534); Houdinière (2); Howell *et al.* (159); Hurel & Mocquot (542); Hussong & Hammer (464); Hussong *et al.* (465).

Irvine *et al.* (54, 55, 56); Itzerott (149).

Jack & Henderson (73); Jack *et al.* (74, 219); Janse (523, 662); Jenness & Herreid (513); Jenness & Palmer (311); Jenness *et al.* (509, 721); Jirgensons (255, 256, 261); Johnson & Gould (547); Jonnard (563) Jørgensen (468); Josephson (344, 427); Josephson & Dahle (345); Josephson & Keeney (455); Josephson *et al.* (426); Judkins (335); Julien *et al.* (695).

Kästli (14, 18); Kästli & Binz (15); Kahane & Lévy (155); Karabinos & Dittmer (116); Kass & Palmer (342); Kaufmann & Lund (644); Kaufmann & Wolf (477); Kay (176, 212); Keeney & Josephson (431); Kelly, E. (162); Kelly, P. L. (215, 216); Keston (238); Kiermeier (669); King, N. (642); King, W. H. & Schouest (410); Klotz (246); Knaysi (654); Knaysi & Guthrie (656); Koenig (389); Koestler (50, 705); Koestler *et al.* (29); Korpáczy & Ersek (639); Kosikowsky & Dahlberg (703); Kosikowsky *et al.* (651); Kothavalla *et al.* (397); Krejci *et al.* (242); Kreker (228); Krienke (423); Krienke & Fouts (463); Kruisheer *et al.* (646); Krukovsky & Guthrie (437, 438); Krukovsky & Herrington (209, 210); Krukovsky & Knaysi (655); Küntzel & Doehner (253); Kugenev (113); Kunkel & Combs (735, 736).

Laki (260); Lampert (511); Lawrence (715); Lea (347, 484, 663, 718); Lea & Gane (726); Lea *et al.* (359); Leeder & Herreid (440); Leighton (752); Leroy & Bonnet (21); Leviton (135, 136); Leviton & Haller (320); Li (291); Liebenthal & Adolph (412); Lighthelm (388); Ling (565); Linneboe (378); Livak (549, 558); Loftus-Hills (369); Loftus-Hills & Conochie (486, 487, 625); Loftus-Hills & Thiel (719); Loftus-Hills & Wilkinson (720); Lotmar & Nitschmann (254); Lucas & Trout (530); Luecke *et al.* (145); Lythgoe (173, 399); Lythgoe & Rosenthal (222); Lyons (323).

Maass (125); MacDonald (383, 395, 619); MacGregor (382); Mangold (665); Marquardt (531); Martin *et al.* (510); Massart & Vandendriessche (163); Mazé (204); Mazé & Mazé Jr. (205); McClymont & Paxton (20); McDowall (371); McDowall *et al.* (471, 472); McDowell (586); McMeekin & Warner (288); Mecham & Olcott (262); Mellander (244, 264); Mellon *et al.* (266); Menefee (740); Mennicke (622, 623); Meuron (631); Miersch & Price (694); Mikič (24); Miller, L. (298, 564); Miller, P. G. & Wise (26); Miller, P. G. *et al.* (751); Mocquot (321); Mohr & Baur (35, 634); Mohr & Eysank (357, 375, 624, 643); Mohr & Hennings (325); Mohr & Mack (641); Mohr & Pasveer (539); Moir (409); Mokrants (404); Monk (394); Montefredine (405); Moore, A. V. & Trout (457); Moore, H. C. & Morrow (32); Morell *et al.* (626); Moriyama (201, 202); Moriyama & Ohashi (203); Mork (13); Morrison & Paige (743); Mosimann (16, 17); Mueller & Mack (449, 450); Muers and Anderson (725); Mühlschlegel (594); Mukherjee *et al.* (3); Mulder (72, 324, 537, 544, 545, 659, 683, 688); Mummery (664); Münchberg & Sulinskas (566).

Narayanaier (421); Nelson (452); Neseni & Korprich (34); Neuberg *et al.* (579); Nicolet *et al.* (109); Nitschmann & Hadorn (268, 272, 273, 274); Nitschmann & Lehmann (243, 263); Nitschmann & Lauener (270, 271, 275); Nitschmann *et al.* (269); Noll & Supplee (35); Nussbaumer & Ritter (640).

Occhialini (322, 406); Olcott & Fraenkel-Conrat (265); O'Loughlin & Ryan (393); Olson, F. C. & Brown (442, 443); Alson, H. C. (470); Olsson (635, 714); O'Shea & Lyons (696); Overby & Madsen (570); Overman (31); Owen (591).

Palmer (310); Parmelee *et al.* (628); Patton & Josephson (453); Pauling (300); Pedersen (617); Peltzer (597); Peters & Trout (317, 318, 328, 329, 330); Peterson *et al.* (208); Pien (230, 618); Pino (132); Platon & Olsson (660); Plumier (147); Pohle *et al.* (148); Polonovski *et al.* (236); Purchase & Reverberi (10); Pyenson & Tracy (722, 745); Pyne (200, 580).

Ram Ayyar & Kothavalla (522); Ramsdell & Whittier (250); Ramsey & Patterson (678); Rangappa (403, 560); Rao *et al.* (257, 258); Rauch & Schloemer (82); Rauschning (596); Rees, H. V. (30); Rees, M. W. (105); Reid (525); Remaley (492); Rensburg, van (9); Richardson & Long (379); Riddet *et al.* (4); Rieman (657); Ritter (632, 689); Robinson & Hlynka (138); Rodkey & Ball (416); Romani (367); Rowland (5, 6); Rowland & Scott Blair (1); Rowland *et al.* (392); Rowlson & Mickle (408); Rudnick (621); Rueda (556); Russell & Dahle (446); Ryan (396).

Saal & Heukelom (432); Sabalitschka (475, 627); Sandelin (42, 128, 140, 142, 251, 252, 312, 313, 374, 536, 543, 708); Sanders & Sager (169, 184, 194); Sarudi (562); Schaffer *et al.* (490); Scharer (179, 190); Schechter *et al.* (612); Schein & Berg (120); Schellhase (233); Schloemer (81, 652, 672); Schloemer & Bleyer (234); Schloemer & Catravas (730); Schloemer & Rauch (524); Schloemer *et al.* (506); Schneck & Plottke (559); Schönberg & Milbradt (232); Schulz (59); Schwaibold & Lesmüller (150); Schwarz & Fischer (227, 710); Schwarz & Hagemann (616, 731); Schwarz & Mumm (49, 540, 706, 732); Schwarz & Sydow (231); Schwarz *et al.* (686); Schweigert (106); Seekles & Smeets (139); Seixas (400); Semmons & McHenry (137); Seuss (711); Sirks (61, 685); Sizer (164); Sjöström (153, 170); Sjollema (77); Skelton (314); Skelton & Sommer (316); Smiley *et al.* (702); Smith, E. L. (301, 303); Smith, E. L. & Coy (302); Smith, G. A. (414); Snyder & Sommer (338); Sörensen (44); Sörensen & Sörensen (129); Solntsev (483); Sommer (309); Spaght (380); Spremull (297); Sproule (53); Stamberg & Bolin (739); Stanworth (750); Stas (144); Steel (331); Stevens (451); Stijgeren, van (167); Stiven (178); Støren & Doyle (653); Storgårds (45); Straub & Bever, van (319); Stuart & Martin (551); Stull *et al.* (459); Sundberg & Hultberg (666); Swartling (469); Sylvester *et al.* (667).

Taha El-Katib (48); Tankard & Bagnall (387); Tapernoux (207); Tarassuk & Henderson (213, 447, 448); Terrier (729); Theorell (223, 224, 225); Thibodeau (692); Thiel (43, 363, 638); Thomé (19, 187, 568, 569); Thompson (744); Townley & Gould (156, 157, 608); Tracy & Hoskisson (495); Tristram (104); Trout & Lucas (526, 529); Trout & Scheid (326, 333); Trout *et al.* (430); Turner *et al.* (693).

Van der Burg *et al.* (83); Van der Waarden (460, 461); Van Slyke & Hiller (584); Veinoglou (390); Venn *et al.* (152); Vilegzhanin (206, 681); Vilstrup & Bielefeldt (240); Virtanen & Rautanen (575); Vladesco (582, 583); Voorst (673); Vorhes (630).

Waisman & Petazzo (115); Warner (241); Warner & Polis (239); Webb & Bell (352); Webb *et al.* (350, 365); Whitaker (336); White, A. H. (370, 629); White, A. H. & Gibson (474); White, J. C. D. *et al.* (349); White, W. B. *et al.* (143); Whitman *et al.* (503); Williamson (112, 493); Willstaedt *et al.* (117); Wilson (614); Winnick (123); Winther (78); Woiwod (589); Wokes & Still (572); Worden (235); Wormell & Kaye (278, 279); Wuhrmann & Högl (581).

Yska (28).

Zahrndt *et al.* (58); Zaribnicky (415); Zarins & Brants (606); Zeisset (700); Zeisset & Grossfeld (98); Zilliox *et al.* (600); Zollikofer (51, 80).

PART II. RHEOLOGY*

Spöttel & Gneist(1) have measured the viscosity of milk from the cow, goat and ewe in a Höppler rolling ball viscometer at temperatures from 10 to 50° C. The viscosity of cow's evening milk is higher than that of morning milk, nor can the differences be accounted for by differences in fat content. Holding milk at 3–4° C. causes a slight increase in viscosity, part of which is independent of fat changes since it occurs in skim milk. Unlike shearing in a capillary viscometer, the rolling-ball technique does not seem to reduce viscosity as a result of repeated tests. Correlation coefficients are given relating viscosity to dry matter, protein, fat content, etc.

Caffyn(2) gives a brief survey of the hydrodynamic and thermodynamic problems involved in H.T.S.T. pasteurization. Bell, Curran & Evans(3) study the effects of time of sterilization and of temperature on the properties of evaporated milk. Comparisons were made between slow sterilization (115° C. for 18 min.) and H.T.S.T. (135° C. for 30 sec.). The effects of fore-warming and varying storage conditions were also studied. The results may be very briefly summarized by saying that slow sterilizing makes for better rheological conditions during storing, whereas H.T.S.T. has advantages with respect to colour and flavour. Deysher, Webb & Holm(4) also make a study of the viscosity of evaporated milk.

It is comparatively rarely that a rheological study of dairy products forms the subject of papers of first-class academic interest, but Eilers & Korff(5) prove a notable exception in their studies on the viscosity-concentration and electro-viscous effects in skim milk. Skim milk is almost a true (Newtonian) fluid, but it is also to be regarded as a polydisperse system for which the usual voluminosity equations are hardly applicable. Qualitatively, however, there is little doubt that pasteurization increases the voluminosity. A study is also made of evaporated and condensed milk and of the thickening of the latter on storing. Both capillary and rotation (MacMichael) viscometers are used.

Jack(6) studies temperature changes during the melting of butterfat. The heating curves show abnormalities presumably caused by different fractions melting at different temperatures. It is suggested that the texture and churning time of butter are much influenced by these differences. Jack & Brunner(7) have extended this work and give a formula from which the percentage of solid fat can be calculated from the amount of heat per g. of fat required to raise the temperature to that value at which all the fat is melted.

A number of new methods have been described for measuring the firmness of rennet curd. Rowland & Soulides(8) measure the displacement of a light tray suitably loaded and resting on the surface of the curd for a standard time. This technique has the advantage that the firmness so determined is a direct measure of the rigidity modulus of the gel subject to the usual approximations.

Spur & Wolman(9) have developed the Chambers-Wolman test in which formalin-hardened curd is sieved through a series of sieves of standard mesh, an arbitrary formula defining the 'curd number'.

Hastings & Erekson(10) study the relations between chlorine and catalase contents of milk and curd tension. Marquardt(11) studies soft-curd milk, and Marquardt & Denniston(12) report on the curd strength of evaporated milk. Storrs(13) has shown that curd tension and curd surface-area tests are independent factors showing no significant correlations. The effects of homogenization and pasteurization on curd tension was investigated by Kelly(14).

Rogers & Sanders(15) give a good summary of work on rheology of cheese and curd up to 1942 and describe three apparatuses: (i) a complicated version of Hill's curd-o-meter, (ii) an 'elasto-meter' working on the principle of the Chopin extensimeter used for flour doughs, which measures the deformation produced by air pressure exerted under a disk of curd, (iii) a 'plastometer' in which cheese is forced through a jet by increasing water pressure.

Schwarz & Fischer(16) measured the consistency of a variety of cheese types in a Höppler consistometer in which a small sphere is forced through the cheese by means of pressure applied

* References up to approximately the end of 1947.

by a weight on a lever arm, a series of pressures being used. Like most plastic materials, cheese shows a fall in consistency with increasing stress, and there is also a partly reversible softening produced by the shearing which the authors described as thixotropy. Elastic compression moduli are measured by loading cylinders and a cone hardness tester is also used.

Koestler (17) has extended considerably the work of which the preliminary papers were mentioned in the last Review. Perhaps the most important points made are the surprisingly large variations in rheological properties in samples from different parts of the same cheese and the relationships between consistency and Ca/P, Ca/N and P/N ratios. Dorner & Ritter (18) have studied the distribution of size of particles of curd in Emmental cheese.

Scott Blair and his colleagues have continued their work on the rheological properties of Cheddar and Cheshire curd at various stages of manufacture and of finished cheese. Scott Blair, Coppen & Dearden (19) made cheese under various controlled conditions, the consistency at pitching being found to be the most important. The body and quality of the cheese were afterwards judged both by experts and non-experts. Cheese-makers are found to show greater self-consistency than do non-experts in grading cheese. With acid-curd cheese, experts and non-experts differed but little in the extent to which their judgements agreed with rheological tests, whereas in sweet-curd cheese, experts were definitely superior. This was ascribed to the fact that experts judge quality as a whole and are not good at judging separately such factors as body and spring. In sweet-curd cheese, the rheological properties were more highly correlated with one another than is the case with acid cheese.

From 1942 onwards. experiments were done each summer in commercial factories in which cheese was made under conditions controlled by rheological tests, the effects of quantitative variations in these conditions on the rheological properties of the green cheese and its quality as judged by experts being statistically studied. The results are reported by Coppen (20), Caffyn (21) and Baron (22).

Mulder (23) has compressed cylinders of cheese between parallel plates. The experimental methods are similar to those first used by Davis in 1937. The deformations under constant load (uncorrected for changing cross-section of test-piece) are divided into recoverable and non-recoverable parts. From the latter, which are found to be proportional to the time under load, viscosities are calculated. For long-time loading (10 min.), the total, elastic and plastic deformations are all proportional to the load. Elastic moduli and relaxation times are also calculated.

As an empirical measure of 'Unyieldingness' (Stugheid) Mulder takes the pressure in g./cm.^{2} ÷ twice the total strain produced in 10 sec. Subjective judgements of elasticity depend on the immediate elastic strain rather than on the relaxation times which are ~800–3000 sec. Rupture loads are also measured.

Caffyn (24) has made a study of the principles involved in the design of instruments for increasing the load on cylindrical test-pieces of cheese and other materials by means of levers in such a way that the load per unit area on the sample remains approximately constant. Data obtained at constant pressure are much easier to handle when complex materials are being tested.

Scott Blair & Coppen (25) have continued their study of the psycho-physical processes involved in judging firmness of cheese-like materials by handling, and have shown the value of the use of fractional differential equations in this connexion. On the manufacturing side Whitehead (26) has shown that a certain type of openness of texture in cheese is eliminated by the use of hydraulic in place of hand presses.

Bell & Sanders (27) have studied the behaviour of cream in a rotation (MacMichael) viscometer and shown that the use of fresh milk containing small unclumped fat globules and low-storage temperatures produces a stable emulsion of good body when thawed after several months' storage. Decker & Reid (28) have studied different mix compositions and methods of processing in relation to texture, structure and stability of ice-cream.

Most of the work on rheology of butter during the period under review has come from Holland. An exception is found in three papers by Dolby (29), who has studied methods for measuring 'spreadability'. Dolby does not care for Scott Blair's method, using the compression of cylindrical test-pieces, since he finds the butter samples inclined to crumble. He prefers to measure the resistance to cutting by means of a wire ('sectility'). The load required to give a constant cutting

rate is found to be approximately proportional to the diameter and to the length of the wire. The test shows a good general correlation with the cylinder compression method. The 'hardness' measured by sectility (in general rheological usage 'toughness' would be a better expression) decreases with increasing temperature. The cutting rate (rate of shear) is related to the applied load by a power law the exponent of which diminishes as the temperature rises. The effects of varying butter-making conditions on sectility were studied. Rapid cooling after pasteurization gave a butter of high sectility.

From Holland, Kruisheer(30) studies the consistency of butter in relation to chemical constitution, and Keestra(31) compares Kruisheer's physical tests for firmness with subjective thumb testing and also studies the effects of stall and meadow feeding on butter consistency.

Mulder(32) has published a long series of reports on the consistency of butter using a penetrometer to measure consistency. The super-cooling of butterfat is studied dilatometrically. Soft summer fat supercools more than hard winter fat. Butterfat should be studied in cream and butter and not as separated fat. The usual view that butter from sour cream is softer than that from unripened cream is a fallacy. The influence of salting on hardness is studied. When butter is warmed and then cooled, clusters of fat crystals are formed which increase hardness and shortness. At high temperatures a recrystallization probably takes place producing a softening.

Mulder & Kleikamp(33) confirm calorimetrically previous findings that churned butter fat crystallizes more easily than fat in emulsion. The specific heat of the former is slightly higher than that of the latter. Adriani & Tamsma(34) discuss practical applications of such investigations to the improvement of the consistency of winter butter.

Peanut butter, though strictly speaking not a dairy product, has much in common with butter, and Vincent & Szabo's work(35) is therefore worthy of mention. A penetrometer is used, the depth of penetration after 1 min. under a standard load at 25° C. being taken as an index of 'spreadability'. Burnett, Roberts & Parker(36) have studied the effect of variations in pH on viscosity of peanut protein, soybean protein and lactic acid casein solutions.

Nitschmann & Guggisberg(37) have studied the optical and rheological properties of casein sols. They are mainly concerned with the electro-viscous effect at low salt concentrations and the change in degree of dispersion of casein at high salt concentrations. Intrinsic viscosity is discussed in relation to Staudinger's law. Materials other than casein—tobacco mosaic virus, ovalbumins, etc.—are also dealt with.

A method developed by Hilker(38) for mayonnaise ('Cultured cream') might prove useful for cream. A graduated aluminium rod, called a 'plummet', having a tapered end, is dropped into the mayonnaise and the depth of penetration is recorded.

The effect of milk products on the heat stability and viscosity of cream-style foods—as determined by a MacMichael viscometer—is studied by Webb & Hufnagel(39).

Milk as a constituent of bread is studied in relation to its heat treatment by Stamberg & Bailey(40) by means of the polarograph and the farinograph. (The latter measures the work required to knead the dough.) Low molecular weight lactalbumin molecules tend to soften doughs, and these are destroyed by the heat treatment of the milk.

Scott Blair, Cowie & Folley(41) and Scott Blair, Cowie & Coppen(42) have extended the work described in the last Review on the rheology of bovine cervical secretions to study the effects of pregnancy on the flow properties in an emptying tube viscometer. For true fluids under constant pressure, $L^2-l^2=kt$, where L is the initial length of the column of secretion, l its length after time t and k is a constant. The curves plotting L^2-l^2 versus t for cervical mucus are, however, non-linear, and their characteristics differ when the samples are taken from pregnant and non-pregnant animals. The lower part of these curves can be extrapolated to give intercepts on the L^2-l^2 axis and these are found to be positive in pregnancy (the secretion being essentially plastic), negative in oestrus (elastic) and almost zero in mid-cycle. Unfortunately the curves do not give an absolutely certain test for pregnancy before about 4 months, and their interest is therefore largely academic.

National Institute for Research in Dairying
University of Reading

G. W. Scott Blair

REFERENCES

(1) Spöttel & Gneist (1942). *Milchw. Forsch.* **21**, 214.
(2) Caffyn (1946). *Dairy Industr.* **11**, 327.
(3) Bell, Curran & Evans (1944). *J. Dairy Sci.* **27**, 913.
(4) Deysher, Webb & Holm (1944). *J. Dairy Sci.* **27**, 345.
(5) Eilers & Korff (1945). *Res. Bull. Dutch Min. Agric.* no. 50, (15) G.*
(6) Jack (1941). *Proc. W. Div. Amer. Dairy Sci. Ass.* p. 68.
(7) Jack & Brunner (1943). *J. Dairy Sci.* **26**, 169.
(8) Rowland & Soulides (1942). *J. Dairy Res.* **13**, 85.
(9) Spur & Wolman (1942). *J. Dairy Sci.* **25**, 409.
(10) Hastings & Erekson (1944). *J. Dairy Sci.* **27**, 337.
(11) Marquardt (1942). *Milk Dlr*, **27**, 72.
(12) Marquardt & Denniston (1942). *J. Dairy Sci.* **25**, 427.
(13) Storrs (1941). *J. Dairy Sci.* **24**, 1041.
(14) Kelly (1941). *Rep. Bur. Dairy Ind. U.S. Dep. Agric.* p. 47.
(15) Rogers & Sanders (1942). *J. Dairy Sci.* **25**, 203.
(16) Schwarz & Fischer (1942). *Milchw. Forsch.* **21**, 110; *Dtsch. Molkereiztg.* **63**, 448.
(17) Koestler (1941). *Milchw. Forsch.* **21**, 62.
(1941). *Annu. agric. Suisse*, **42**, 598.
(1942). *Mitt. Lebensm. Hyg.* **33**, 33.
(1942). *Schweiz. Milchztg*, **68**, 344, 348.
(1943). *Annu. agric. Suisse*, **44**, 266.
(18) Dorner & Ritter (1942). *Schweiz. Milchztg*, **68**, 113.
(19) Scott Blair , Coppen & Dearden (1942). *J. Dairy Res.* **13**, 73.
(20) Coppen (1943). *Dairy Industr.* **8**, 488.
(21) Caffyn (1945). *Dairy Industr.* **10**, 257.
(1946). *Dairy Industr.* **11**, 249.
(22) Baron (1947). *Dairy Industr.* **12**, 548.
(23) Mulder (1945). *Rep. Hoorn Exp. Sta.* no. 51 (12) C.
(24) Caffyn (1944). *J. Sci. Instrum.* **21**, 213.
(25) Scott Blair & Coppen (1943). *Amer. J. Psychol.* **56**, 234.
(26) Whitehead (1946). *N.Z. J. Sci. Tech.* **27**, 406.
(27) Bell & Sanders (1946). *J. Dairy Sci.* **29**, 213.
(28) Decker & Reid (1942). *J. Dairy Sci.* **25**, 726.
(29) Dolby (1942). *J. Dairy Res.* **12**, 329, 337, 344.
(30) Kruisheer (1942). *Rep. Leyden Exp. Sta.* Sect. 8.
(31) Keestra (1940?). Brochure from Zuivel-Kwaliteitscontrole Bureau, Amsterdam.
(32) Mulder (1939). *Rep. St. agric. Exp. Sta. Hoorn*, no. 45.
(1940). *Rep. St. agric. Exp. Sta. Hoorn*, no. 46.
(1941). *Rep. St. agric. Exp. Sta. Hoorn*, no. 47.
(1941). *Versl. landb. Onderzoek.* **100**, 919.
(1945). *Rep. St. agric. Exp. Sta. Hoorn*, no. 51 (3) C.
(1947). *Rep. St. agric. Exp. Sta. Hoorn*, no. 52 (6) C.
(33) Mulder & Kleikamp (1946). *Rep. St. agric. Exp. Sta. Hoorn*, no. 51 (C).
(34) Adriani & Tamsma (1946). *Versl. landb. Onderzoek.* no. 52 (1) G, p. 1.
(35) Vincent & Szabo (1947). *Analyt. Chem.* **19**, 655.
(36) Burnett, Roberts & Parker (1945). *Industr. Engng Chem.* (Indust. ed.), **37**, 276.
(37) Nitschmann & Guggisberg (1941). *Helv. chim. Acta*, **24**, 434, 574.
(38) Hilker (1947). *J. Dairy Sci.* **30**, 161.
(39) Webb & Hufnagel (1946). *J. Dairy Sci.* **29**, 221.
(40) Stamberg & Bailey (1942). *Cereal Chem.* **19**, 507.
(41) Scott Blair, Cowie & Folley (1942). *J. Physiol.* **101**, 11*P*.
(42) Scott Blair, Cowie & Coppen (1942). *Nature, Lond.*, **149**, 609.

* English version in Eilers, Saal & van der Waarden (1947), *Monographs on the Progress of Research in Holland during the War*, no. **12**. New York and Amsterdam: Elsevier Publ. Co., Inc.

(*MS. received for publication* 29 *July* 1949)

land